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SOFTENING OF THE SEEDS OF MELILOTUS ALBA

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(WITH PLATES IV, V AND FOUR FIGURES)

Historical summary

Sweet white clover, *Melilotus alba* Desrousseaux, for a long time known as a prolific weed, has in recent years found a definite place among field crops, and is now rather extensively cultivated. In common with other legumes, it produces hard seeds which remain unswollen when placed under germinating conditions. It is only when the coats have become permeable and softening occurs that germination is possible, and as such a condition may be indefinitely delayed, hard-seededness is undesirable from the farmer's standpoint.

This condition of hard-seededness varies in degree from one species to another, and from one sample to another within a species. HARRINGTON (**14**) worked with the seeds of the small-seeded legumes, of which the greater part were field crop plants. Investigating twelve species, he reports hardness varying from 98 to 0 per cent, the percentages being high in the alfalfas and clovers. Since the mature seeds of *M. alba* have particularly impermeable seed coats, this species has been chosen as the subject for this investigation. In this seed, the layers of tissue surrounding the embryo are of the same general character as those in other legumes (fig. 1).

Immediately outside the embryo lie a few layers of endosperm cells, including an aleurone layer. Outside these, in the seed coat proper, are cells with large lumina, called nutrient tissue by TSCHIRCH (**31**), and in which YOUNG (**33**) found starch when the

immature seed was developing. Next come the osteosclerid cells with their peculiar inwardly directed buttresses, and the columnar Malpighian cells, so named by TOZZETTI (30). These last cells form a columnar epidermal layer, and have caps visibly different in appearance from the secondary walls below. At the junction of the caps and these walls is the light line. The caps are imbedded in a

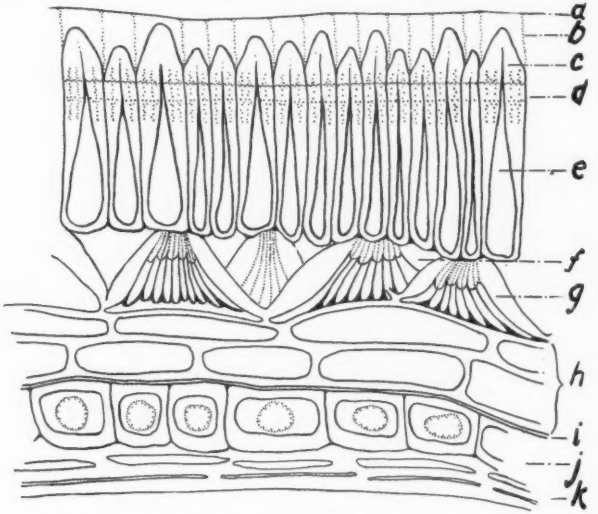


FIG. 1.—Radial section of seed coat: *a*, cuticle; *b*, subcuticular layer; *c*, caps of Malpighian cells; *d*, light line; *e*, lumen of Malpighian cell; *f*, intercellular space; *g*, osteosclerid cells; *h*, nutrient layers; *i*, space between seed coat and endosperm; *j*, aleurone layer; *k*, inner layers of endosperm; camera lucida drawing; ×1613.

superficial matrix known as the cuticularized layer and covered externally by a cuticle. Since the term "cuticularized" is misleading in its allusion to the composition of the layer, the term "subcuticular layer" will be used in this paper. Apically the lumen becomes small, being encroached upon by flangelike thickenings of the walls, so as to present in longitudinal sections the appearance of a number of canals or pores.

The chemical composition of the various regions of the Malpighian layer has been investigated in *M. alba* and related species by a number of investigators. The cuticle and subcuticular layer were investi-

gated by PAMMEL (27); WHITE (32) combined both layers under the term cuticle; REES (29) described them as mainly hemicellulose. COE and MARTIN (5) are the only workers who have described these cells with any detail, and they refer to the cuticle as thin and pervious to water, and to the subcuticular layer as "particularly free from cutin" and "readily permeable to stains." PAMMEL mentions the conelike caps but does not describe their composition; WHITE illustrates them; REES refers to them as being cutinized; and COE and MARTIN conclude that the difference between the caps and the subcuticular layer is probably a "difference in density rather than a difference in composition," and further state, "that in the region above the light line, there is only a trace of cutin or suberin, but a considerable amount of pectose." They watched the action of sulphuric acid on these layers, and found "that the action of the acid was rapid, destroying the cuticle, the cuticularized layer and cones in about five minutes." REES states that the walls below the caps and the light line are mostly hemicellulose; and all who describe their composition refer to their positive reaction with cellulose reagents.

JUNOWICZ (16) reviewed the previous work on the subject, and concluded from his own work that a series of uniform papillae and folds gave luminous points because of differently imbibing cellulose lamellae; while BECK (2), unable to observe these structures, believed that chemical modifications existed. The explanations of most modern workers have been in agreement with one or the other of these hypotheses, but no commonly accepted explanation has been offered. COE and MARTIN decided that it was caused by a special substance, callose, and also a substance "chemically different from all other parts of the seed coat, or else so different as to resist the attack of the reagents." LUTE (18) described the phenomenon in *Medicago sativa* as an "optical illusion," without reference to microchemical evidence.

Hard-seededness was noted long ago, and NOBBE (26) suggested that the phenomenon was caused by a waxy layer over the seeds. PAMMEL found the cuticle thicker in those seeds in which germination proceeds rather slowly. WHITE explained the impermeability of hard seeds as the result of a thick cuticle, including under this term

both the cuticle and the subcuticular layer. REES found that the hard seeds of *M. alba* and other related species became soft when the upper walls of the Malpighian cells became separated from each other. COE and MARTIN believed that the light line substances formed the impermeable region of the seed coat, and were pierced by closed canals, which were opened by the action of sulphuric acid, thus permitting water to pass. Recently LUTE has stated that alfalfa seeds become permeable when the tips of the Malpighian cells are planed through.

MATTIROLO and BUSCALIONI (**19**) first described the functions of the hilum, the micropyle, and the fissure of the strophiole. They held that variations of humidity were the cause of structural and volumetric modifications, resulting in a movement of air through the micropyle which was beneficial to the embryo just previous to germination. In the alfalfas and clovers these structures have not been regarded as related to the state of hardness in a seed; and until lately all workers have agreed with COE and MARTIN on this point.

Recently ESDORN (**9**) has been interested in these structures in *Lupinus luteus*, and is convinced that they are related to the "hartschaligkeit" of that seed in storage. She regards the state of hardness as a reversible condition, the changes from hard to soft and from soft to hard being governed by conditions of relative humidity. She claims that the whole surface is permeable in soft seeds.

Hard seeds possess long life, although soft seeds of the same species rapidly lose the power of germination. The extensive research of BECQUEREL (**3**) and EWART (**10**) showed that hard seeds are macrobiotic. EWART reported that a sample of *M. alba* which had been stored in a laboratory for 77 years germinated 18.2 per cent following softening.

While soil is not a favorable place for storage, the work begun by BEAL in 1879, and reported by DARLINGTON (**7**) and GOSS (**11**), has shown that seeds can survive long periods when buried in the earth. In GOSS's experiments some seeds of red clover still remained hard after 20 years in the soil. In one lot 15.5 per cent germinated when clipped. It is this potentiality for germination after several years that makes hard seeds lying in the soil a handicap to future crops.

Much work has been done in an effort to soften hard seeds. It was

early discovered that abrading or scarifying the coat would render impermeable seeds permeable to water. A limited amount of scarification occurs during threshing, and machines have been invented to accomplish this purpose more completely. This method has been useful, and much scarified seed has been sown. Scarification injures seed more or less, of course, and considerable work has been done to determine its limitations. One disadvantage is the short life of scarified seed in storage. Thus GRABER (**12**) found that with 2–4.5 years' storage the average immediate germination of unscarified alfalfa samples was 71.6 per cent and after storage 74.4 per cent. Scarified samples of the same seed gave an average immediate germination of 86.4 per cent, which, after storage, fell to 40 per cent.

Scarified seeds produce damaged seedlings on germination. NELSON (**22-25**) holds that broken seedlings are caused by the unequal restricting pressure of the damaged seed coat upon the swelling embryo; but, whatever the cause, broken seedlings are very noticeable when scarified seeds are germinated. HARRINGTON (**14**) and LEGGATT (**17**) have concluded that as hard, unscarified, threshed seeds softened in the field they became useful in producing a crop. This was of value when the field was used for forage or pasture for more than one year. LEGGATT concluded that hard seeds of *M. alba* have approximately 50 per cent of the value of scarified seeds.

Because of the disadvantages which attend scarification, other means of softening have been attempted. REES (**29**) used ethyl and methyl alcohols of various concentrations, chloroform, and turpentine in an effort to dissolve out impermeable fats and waxes from the seed coat, but with little practical success. Heating has been used with success by EWART (**10**), MÜLLER (**21**), HARRINGTON (**14**), and LUTE (**18**). Prolonged heating has been reported as decreasing the vitality of the seed.

HARRINGTON reported that seeds treated with temperatures alternating from below 10° to above 20° C., and also seeds placed in water and alternately frozen and thawed, increased in germination percentage but also in mortality rate. MIDGLEY (**20**) found that an initial freezing produced softening of alfalfa seeds, but subsequent freezings had no effect in reducing impermeability. In discussing the effect of low temperatures on seeds, BUSSE (**4**) refers to the probable

formation of tiny cracks in the impermeable membrane as the result of the different coefficients of expansion of various parts of the seed. He shows that softening of alfalfa and sweet clover seeds follows temporary immersion in liquid air or cooling with carbon dioxide snow. Germination was increased in sweet clover from 64 to 94 per cent by the use of liquid air, and the use of carbon dioxide is recommended for the inexpensive softening of alfalfa seed.

Other methods have been used successfully in softening of seeds, although so far their use has been restricted to small lots. Sulphuric acid was first used by HILTNER (**15**); and since that time WHITE (**32**), REES (**29**), and COE and MARTIN (**5**) have used it in investigating the histological problems of impermeability. Caustic soda is mentioned by EWART (**10**) as a reagent for softening, but it is recommended only as of assistance in the identification of dead seeds. The effect of pressures on seeds has been discussed by DAVIES (**8**), who states that at the end of 30 days following the subjection of *M. alba* seeds to a pressure of 2000 atmospheres, germination rose from 25 to a maximum of 90 per cent after which it fell progressively with time.

Methods and material

The material used was the seed of *Melilotus alba*, collected in the summers of 1927 and 1929 at Landsdowne and Hawkestone, Ontario; and in order to reduce accidental scarification to a minimum, hand harvesting was employed. The seed was stored in containers in the laboratory until needed for the experiments. Only plump, mature seeds of good color were used, after being freed from the pericarp by rubbing between the hands.

The thin sections necessary for accurate study of the minute structures were cut dry by the Thomson-Jeffrey microtome. Fresh dry seeds, glued to wood blocks with Lepage's glue or celloidin, supplied sections 4 to 5μ thick, and ordinary methods of imbedding, with the possible resulting alterations in the seed coat, were eliminated. A slightly moistened brush was used to prevent the sections from rolling up, and to place them on a clean slide in water. With the aid of a binocular microscope, the embryos were dissected away from the seed coat and removed, together with the starch and débris from the endosperm, by means of blotting paper.

Dyes have proved useful although unreliable agents in the determination of the composition of the tissues, as HARLOW (13) has shown. In critical work, chemical and microchemical examination became a regular procedure.

Mounts were satisfactorily made in either glycerin or glycerin jelly, prepared according to Kaiser's formula. Distortion was avoided through the use of the former, and this was always used when permanent mounts of dry prepared material were required. ZIRKLE's (34) method of imbedding in paraffin was followed when the serial sections for morphological study were required.

Histology and microchemistry

Inside the seed coat, immediately surrounding the embryo, is the endosperm. The cells of the inner layers have thick walls which almost occlude their lumina. On the outer surface is a layer of cells possessing larger lumina and containing aleurone grains (fig. 1). Outside this tissue, and separated from it, are the inner layers of the seed coat. The inner part of the seed coat consists of a few layers of thin-walled cells with large lumina, the nutrient layer. Around this tissue is found a single layer of the conical broad-based osteosclerid cells, separated by intercellular spaces at their tops. The lumen of these cells is broad at the base, narrow at the top, and is given a curious appearance by the vertical flangelike projections of the secondary wall into the interior.

The cells of these layers are easily permeable. Their walls swell greatly when immersed in water. They disappear upon treatment with hydrochloric acid and ammonia,[1] which is a characteristic reaction for pectic compounds, and the absence of other more refractory materials. The presence of these compounds is further indicated by pink staining with ruthenium red and violet staining with methylene blue. The entire absence of cellulose is indicated by the complete and ready hydrolysis with hydrochloric acid and ammonia, and the presence of cellulose-like compounds through partial hydrolysis with copper oxide ammonia. Figure 5 shows a section treated with this

[1] The material is covered with 2% hydrochloric acid and kept between 50° and 90° C. for 20 minutes. It is then washed and covered with 2% ammonia, heated as before, and finally rewashed. This treatment hydrolyzes insoluble pectic compounds present to pectic acid, which, in the presence of ammonia, forms a soluble pectate.

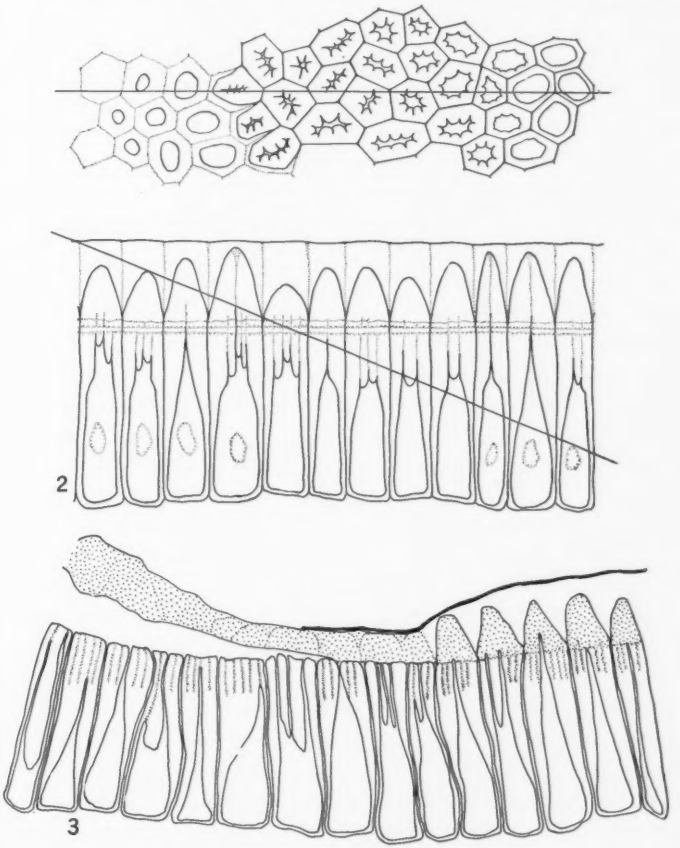
reagent where the walls are thinner than normal. Chlorzinc iodine leaves a trace of blue in these layers, while dilute sulphuric acid hydrolyzes them so completely in a few minutes that no staining is visible when a dilute iodine is added; but no blue staining appears in these regions when sections are treated with iodine and sulphuric acid in various concentrations. The tissues quickly disappear unless very dilute acid is used. These tests indicate a primary wall of pectic substance with secondary walls largely of hemicellulose.

Although near the apex the flanges of the Malpighian cells are closely pressed together, the lines of demarcation are still distinctly visible. JUNOWICZ (**16**) referred to these lines and clefts in *Lupinus varius* as canals. They may be seen in section in the upper drawing of figure 2. The apices of these cells project as cones into an imbedding matrix, the subcuticular layer, which is covered externally by a thin cuticle. The cuticle is insoluble in hydrochloric acid followed by ammonia, and also in concentrated sulphuric acid, and may often be observed floating free when whole seeds are treated with the latter reagent. It is slightly soluble in chromic acid and little affected by strong alkalies. It is so thin that clearly visible staining occurs only with chlorzinc iodine, when it appears yellow.

These reactions show that this layer is composed of cutin, porous enough to permit sulphuric acid, hydrochloric acid, ammonia, and other liquids to penetrate it, as shown by the fact that they dissolve away the matrix investing the caps in uninjured seeds. The cuticle may be removed by maceration when the supporting subcuticular layer is dissolved by hydrochloric acid and ammonia, or through the action of ammonia alone. Similarly, when the seeds were incubated at 37° C. with three successive, active maize cultures of *Clostridium acetobutylicum* Weizmann, the cuticle was removable, owing to the digestion of this underlying layer. Such treatment does not result in the softening of hard seeds to any greater extent than treatment with water at the same temperature. The actual softening at 37° C. is slight, even when seeds have been left incubating in cultures of the bacillus for more than four months. The cultures had, of course, become inactive long before the expiration of this period.

In sections, the subcuticular layer stains violet with methylene blue and pink with ruthenium red, corroborating the evidence of its

pectic nature as already described. With chlorzinc iodine the layer turns slightly blue, the only indication of a cellulose-like compound



FIGS. 2, 3.—Fig. 2 (above), partial tangential and true radial sections of seed coat; lines indicate relation of sections to one another; camera lucida drawing. Fig. 3 (below), radial section of seed coat of sulphuric acid-treated seed; camera lucida drawing; ×753.

obtainable. Figure 5 shows a section treated with copper oxide ammonia in which this matrix appears unchanged, which would not be the case were any considerable amount of cellulose or hemicellulose

present. Under the sulphuric acid and iodine tests for cellulose and hemicellulose, the matrix entirely disappears.

From these tests it would appear that the subcuticular layer is a pectic matrix, with probably a trace of hemicellulose or cellulose too small to remain in position when the supporting pectic material is removed. With a subcuticular layer of this kind it is evident that the matrix does not possess the impermeable character which RALEIGH (**28**) thought was the cause of hardness in the seeds of *Gymnocladus dioica*. RALEIGH has suggested the exclusion of water by pectic substances. The pectic subcuticular layer is certainly not the agent responsible for this exclusion. It has been removed without affecting the penetrability of the coat, and in experiments to be described later seeds were made permeable with this layer maintained intact. In fact, there is no evidence to show that pectic compounds, wherever situated, possess waterproofing powers.

These results are in conflict with the views of WHITE (**32**) and REES (**29**), agree partially with COE and MARTIN'S (**5**) views of its composition, and give tentative support to their guess that "this layer is probably the primary epidermal wall."

There are, however, two considerations to be dealt with, namely, the relations of the caps to one another and to the middle lamellae of the side walls. If the unmodified pectic layer of the middle lamella is continued between the caps, a pathway for water is provided and the utility of the caps as waterproofing elements becomes negligible. Figure 5 shows a section from which the cellulose secondary walls have been dissolved by copper oxide ammonia, and the caps are seen to be in contact at their shoulders. In addition, close examination of thin sections stained with methylene blue fails to reveal the presence of pectic substances separating the shoulders of the caps. While monochromes inadequately illustrate such subjects, figure 6 fairly represents the absence of lamellae at these points. In order to obtain a photomicrograph sharply in focus, and to show the red stained substances in the utmost contrast, a nearly monochromatic green filter combination was used (Eastman M filters B & H).

Maceration of the Malpighian layer into the separate cell components may be accomplished by treatment with hydrochloric acid and ammonia, or with ammonia alone. Two stages in the maceration

are readily observed under the microscope. The former stage occurs after sufficient time has elapsed for the solution of the subcuticular layer and the middle lamellae, when, if the cover slip is gently tapped, it is found that the lower ends of the Malpighian secondary walls are separate from each other, while the bases of the caps still remain attached. The latter stage occurs on further tapping, when the cells separate completely. The invariable coherence of the caps, after all pectic substances have been dissolved away, militates against any idea of a continuity of pectic substance between the two regions.

The single Malpighian cells, after maceration by this method, measure up to 40μ in length and to 10μ in diameter. Since they are polygonal in section, and appear as pointed cylinders, there is much refraction and observation is difficult. To obtain clear views it is necessary to have a bright source of illumination, giving a parallel beam large enough to fill the aperture of the condenser. Oil between the slide and a nearly wide-open condenser help, as do a good oil immersion objective and a suitably corrected ocular, set at the proper separation distance. These conditions of illumination were not entirely fulfilled in figure 7, as it was desired that the unstained cell might show greater contrast, and give (so far as a photomicrograph can in one plane) a reasonable view of the peculiar cylindrical structure.

In unstained sections there is always a clear line of demarcation between the caps and the layers above, which suggests a difference in composition. The possibility that these caps may be instrumental in excluding water was suggested by the work of REES (**29**) with sulphuric acid, and of L JTE (**18**) with scraping and soaking tests. Special attention was therefore paid to this region, with the idea of establishing the presence or absence of an impermeable substance.

Whole seeds were soaked in sudan III, but it was found that the subcuticular layer was comparatively impermeable to this stain. After six months' soaking in the dye, which was changed from time to time, the caps were clearly stained although the membranes outside remained clear. It was then found that when the cuticle and subcuticular layer were removed, staining was much more rapid. Methylene blue penetrated quickly and stained the caps a blue-green, indicating the presence of cutin or suberin. The reaction of

whole seeds with crystal violet provided confirmatory evidence. For the purposes of identification, Canadian alfalfa and clover seeds entering the United States are required by law to contain a small percentage of seeds stained purple with gentian violet (crystal violet with lower homologs). The staining is very rapid, the caps being the only parts deeply colored. CONN (**6**) recognizes crystal violet as a valuable histological stain, replacing haematoxylin, but makes no mention of its ability to penetrate and stain fatty substances. However, a solution of crystal violet in water poured over a layer of lard in a test tube penetrated and deeply stained the fat.

Specific fat-staining reagents have proved almost useless in staining thin sections. Sudan III staining was not seen in the caps, except when sections were made from seeds which, through immersion in the dye for many months, had become Chinese red in color. The presence of suberin, however, was indicated by slight reactions with sudan IV, methylene blue, and chlorzinc iodine. The last color occurred with the same reagent both before and after treatment with concentrated potassium hydroxide at 100° C. With iodine, a yellowish brown appeared in untreated sections and in those treated with sulphuric acid of various concentrations. The absence of blue coloration with sulphuric acid and iodine indicates that pure cellulose is not present, and the greenish blue obtained with chlorzinc iodine indicates rather the presence of phellonic acid, a constituent of suberin.

While concentrated sulphuric acid softens the caps of the Malpighian cells, it does not destroy immediately the impermeability of the cap layer. Seeds have remained in the concentrated acid for 40 minutes without becoming permeable when transferred to water. This was possible only when every precaution was taken to avoid rubbing the seeds, while they were transferred to a considerable volume of water, for heat caused softening when small amounts were used. The mere stirring of these impermeable seeds while immersed in water is sufficient to break this layer and to render the coat permeable to water. Figure 3 shows a camera lucida drawing of a section from such a seed.

In figure 9 the finger-edged caps of the Malpighian cells which have been treated with chromic acid are clearly indicated. A similar effect is observed when the tissue is macerated with sulphuric acid.

These peculiar caps also remained intact when single Malpighian cells, obtained by pectic maceration, were treated with copper oxide ammonia which dissolved the rest of the cells. This illustration shows also the shoulders of neighboring caps still in contact. This evidence indicates that the caps of the Malpighian cells are of suberin, tightly appressed at their bases, and implies a very different chemical in the outer layers of the Malpighian cells from that given by COE and MARTIN.

The peculiar structure of the Malpighian cell wall inside the caps was usually examined in sections lightly stained with methylene blue. Other stains, such as cotton red, chlorzinc iodine, sulphuric acid, and iodine gave additional support to the belief that cellulose alone was present, except in the middle lamella. These latter stains were much less satisfactory in determining the shape of the wall. Figure 5 shows a section in which, after treatment with copper oxide ammonia, the secondary walls of the Malpighian cells have been dissolved away, leaving the parts not composed of cellulose intact. On treatment with chromic acid, the primary and secondary walls dissolve quickly, the reaction being more rapid in the thin parts at the base than in the thicker wall toward the top. This disappearance, accompanied as it is by bubbling and the appearance of dark, reduced chromic acid, is characteristic of the reactions which take place whenever cellulose is present. Figure 8 was taken not long after this reaction had commenced, when the dissolution of the cellulose in the Malpighian cell wall had become visible.

These tests leave no doubt that cellulose is the chief constituent of the Malpighian cell wall, although the blue coloration with iodine after treatment with dilute sulphuric acid indicates the presence of a small amount of hemicellulose.

Concentrated sulphuric acid is curiously inactive in attacking this wall, even after the caps and outer layers have been removed by maceration. Figure 3 shows the condition after such treatment. Similarly, when perfectly dry sections are placed in concentrated sulphuric acid, few signs of reaction take place. If water is present, however, hydrolysis occurs at once. All parts below the caps are reduced to an amorphous mass in a few minutes, and show the violet-blue amyloid reaction with iodine. When dilute acid is used, this de-

structive hydrolysis does not take place, although dark blue staining of the intact body walls appears on the addition of iodine.

Extensive effort has been made to identify callose and other substances which differ in any respect from the materials tabulated in table I, and whose reactions have been referred to. No other substances have been found, and it is believed that the light line phenomena are due to other causes.[2]

The opened canals which COE and MARTIN found in the impermeable substances of the light line are normally present, although distinctly visible only when filled with air. These canals are conspicuous in this state, which is well illustrated by their drawings. The canals have been seen many times in sections, in which air has entered the apical and lateral extensions of the Malpighian cell lumen, when sections were cut dry or inadequately protected by liquids. The source of the material made no difference, whether imbedded in paraffin or merely glued to the mounting block.

Certainly air is the cause of this phenomenon, for when it is removed from the Malpighian cell lumen by an air pump and replaced by liquids, the canal-like appearance is no longer evident. Moreover, examination of many oblique tangential sections has always indicated the continuity of the lumen from the cap to the base of the Malpighian cell.

Experimentation

It has heretofore been impossible to determine accurately how the impermeable seed coat differed from the permeable, for lack of means of distinguishing hard and soft seeds in the unswollen condition, since soft seeds could be recognized only after sufficient water had been imbibed to produce marked swelling. In order to compare such soft seeds with hard ones, they have been dried and mounted for hand sectioning, or imbedded in paraffin. After either treatment, there is no assurance that the seed coat retains the histological condition of the soft seed before swelling, owing to expansions and contractions.

The swelling method is useful for determining the proportion of hard and soft seeds in a given sample, but it is useless for predetermining whether a particular seed will or will not imbibe water. It

[2] The writer intends to deal critically with this problem in a future paper.

	Reagent	Malpighian layer: Cuticle	Malpighian layer: Subcuticular layer	Malpighian layer: Caps	Malpighian layer: Middle lamella	Malpighian layer: Secondary wall	Osteosclerid layer	Nutrient layer	Endosperm
Micro-chemical reagents	Ammonia (2%). Chlorzinc iodine	Penetrated Yellow	Digested Gives slight hemicellulose reaction	Insoluble Blue green becoming blue when heated with conc. KOH	Dissolved	Unchanged Dark blues	Tissue separates and disappears Light blues		
	Clostridium acetobutylicum..	Penetrated	Digested	Insoluble					
	Chromic acid...	Soluble	Soluble	Slowly soluble	Disappears	Rapidly soluble			
	Copper oxide ammonia.....	Insoluble	Appears unchanged	Insoluble	Insoluble	Dissolved	Part of secondary walls disappear		
	Glycerin 200°–240° C.....	Apparently unchanged	Apparently unchanged	Apparently unchanged	Apparently unchanged	Apparently unchanged			
	Hydrochloric acid 2% and ammonia 2%.	Penetrated	Digested	Insoluble	Dissolved, cells may be macerated apart	Unchanged	Tissue separates and disappears		
	Sulphuric acid (concentrated)	Penetrated but insoluble	Disorganized and easily macerated	Softened	Apparently unchanged	Apparently unchanged			
	Sulphuric acid (75%) and iodine.....	Yellow and insoluble	Destroyed	Yellow and unchanged	Disappears	Amorphous amyloid blue			
	Sulphuric acid (dilute) and iodine.....	Yellow and insoluble	Unstained and soluble	Yellow and unchanged	.....	Undestroyed blue-stained	Disappears		
	Water.....	Penetrated by hot water	Slightly hydrolyzed	.....	.....	.....	All tissues swell in water slightly moderately considerably		
Dye reagents	Cotton red.....	.....	.....	.....	.....	Stained red			
	Crystal violet...	Stained	Slightly stained	Deep violet	Stained	Slightly stained			
	Light green.....	.....	Pale green	.....	Pale green				
	Methylene blue.	.....	Violet	Green	Violet	Blue	Blue and a little trace of violet at middle lamellae Aleurone grains stain		
	Osmic acid.....	.....	.....	.....	.....	Cytoplasm blackened			
	Ruthenium red..	.....	Pink	Unstained	Pink	Unstained	Whole tissue pinkish, bright at middle lamellae		
	Safranin.....	.....	.....	Pale red					
	Sudan III.....	Unstained	Unstained	Pinkish staining takes time	Unstained	Unstained			
	Sudan IV.....	Unstained	Unstained	Pink					
	Composition...	Cutin	Pectic compounds trace Hemicellulose	Suberin and Phellonic acid	Pectic compounds	Cellulose Hemicellulose(?)	Middle lamellae = Pectic compounds Secondary walls = Hemicelluloses		

was found that this could be accomplished through the use of osmic acid (osmium tetroxide). This is readily reduced through brown shades to black amorphous osmium when in the presence of active oxygen acceptors, such as the unsaturated carbon compounds, the unsaturated fatty acids, and olefines. VON ALTMANN (1) has shown that the deep black staining occurs only in the presence of these compounds, and never with the saturated fats.

On treating clover and alfalfa seeds with a fresh 1 per cent aqueous solution, rapid penetration through any opening or permeable area takes place, as is evident from the blackening in the lumina of the Malpighian cells where the osmic acid has been reduced by the unsaturated compounds present. The black stain develops in those cells around the openings in the seed coat before sufficient water has entered to produce visible swelling. After longer exposure to the solution, swelling is noticeable, proceeding from the blackened areas until the whole seed is affected. By a short osmic acid treatment it was thus possible, not only to determine beforehand whether a particular seed would or would not imbibe water, but also to identify the particular areas of the seed coat which were permeable, and this without allowing the penetration of any liquid beyond the Malpighian layers of cells in the seed coat.

When *M. alba* seeds were placed in osmic acid, it was found that blackening always occurred at the hilum, where some funicular tissue remains attached. With soft seeds it occurs at other places as well. The black-staining tissue at the hilum consists of the remains of the parenchyma-like abscission tissue which has finally separated the seed from the funiculus. Other fat reagents stain this tissue in a similarly definite fashion and support the opinion that unsaturated fatty substances are present.

Osmic acid has a high vapor pressure, and in a 1 per cent solution is very active, both in evaporation and in rapid penetration of material. To avoid weakening the reagent and to prevent escape of its fumes, the staining was ordinarily carried out in covered Syracuse watch glasses.

These peculiar stain reactions with osmic acid permit classification of soft clover and alfalfa seeds according to the kind of permeable area into the following groups: (1) seed scarified, showing ir-

regular blackening around holes, cracks, and abrasions in the seed coat; (2) seed softened by sulphuric acid treatment and subsequent maceration, presenting irregular permeable areas of variable size; (3) seed showing unlocalized permeable areas as the result of natural processes or the localized application of heat; (4) seed showing only a small permeable area at the strophiole, as the result of natural forces or heat.

Scarified seed

Threshed and scarified seeds which have been treated with osmic acid show blackening in the cells of the Malpighian layer bordering on breaks in the seed coat produced by scarification. When the swelling of these seeds is carefully observed, it is seen to proceed from these damaged areas; and it is from these that the embryo emerges during abnormal germinations which produce the damaged and weakened seedlings reported by Nelson (**22**–**25**).

In addition to those showing actual scarification, many soft seeds in commercial samples show only the blackened area at the strophiole. These will be referred to later.

Sulphuric acid treatment

Various sized permeable areas are produced on seeds by sulphuric acid; and under laboratory conditions, several kinds of peculiarities are readily observable by the use of osmic acid staining.

Hard seeds covered with concentrated sulphuric acid from 5 to 40 minutes, macerated by stirring, and washed thoroughly and rapidly to avoid visible imbibition of water, when immersed in an osmic acid solution, develop blackening rapidly; and subsequently swelling in the region of the stained areas is evident. Portions of the cuticle may be seen which were not removed by stirring at the end of the acid treatment; the remainder of it is to be found in the acid bath. The blackening spreads rapidly from those large areas which have lost their caps, and soon the whole seed is black, covered here and there with patches of the unstained caps.

Figure 4 shows seeds which have been treated for 20 minutes in sulphuric acid and then allowed to swell in an osmic acid solution. They have expanded, bursting the coats. Three distinct layers are to be noted: the finely stippled areas are the remaining masses of

caps still adhering to the rest of the coat; the black, remaining part of the Malpighian cell layer; and the coarsely stippled areas, the visible parts of the swollen layers lying between the Malpighian layer and the embryo. Figure 3 shows a camera lucida drawing of a section from a seed which had been treated for less than 40 minutes in sulphuric acid. Three regions are visible, showing different stages in the process. On the right are five cells whose form is as yet unchanged, although undoubtedly some hydrolysis has taken place in the subcuticular layer. In the center the subcuticular layer has disappeared and the caps have softened into an amorphous mass, which,

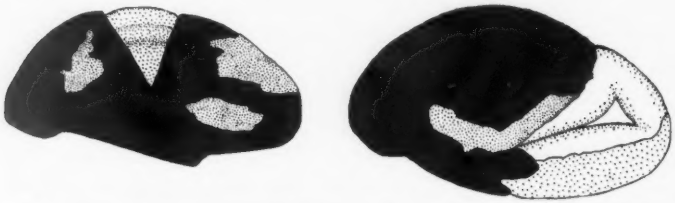


FIG. 4.—Sulphuric acid-treated seed swollen in osmic acid solution; finely stippled areas indicate disorganized caps; medium stippled areas indicate inner tissues of seed coat; lightly stippled areas indicate embryo; ×13.

however, is still in close contact with the remainder of the cells. To the left the cuticle has gone and the softened mass of caps has pulled free from the lower parts.

Seeds which were impermeable in water following a 40-minute treatment with concentrated sulphuric acid were carefully washed, dried, mounted, and sectioned. The condition throughout the seed coat was found to be as illustrated in the central part of figure 3. This result also conflicts with that of COE and MARTIN. Most certainly the condition they describe would be observed in seeds which had not received extremely careful handling, for the layer of disorganized caps resulting from acid treatment is soft and fragile. No permeable acid-treated seed has shown this layer intact.

UNLOCALIZED PERMEABLE AREAS

These unlocalized permeable areas, readily identified by osmic acid staining, are found now and then on wrinkled brown seeds, and on permeable seeds which have been tested following heating in thin

glass vessels over open flames. The appearance of these spots is usual when the seeds are heated dry, and occurs occasionally when the seeds are immersed in water and resting on the bottom of the container. Softening of this character has never been found when the vessel was heated in a water bath or in a constant temperature oven. On brown seeds these areas have been found only when desiccation was sufficiently advanced to wrinkle the seed coat, and on normal impermeable seeds only when small areas of the seed coat had been subjected to high temperatures. These facts suggest that strains separate the caps of the Malpighian cell layer and so permit the movement of liquids and gases.

Brown seed coats are always associated with plasmolyzed Malpighian cells, yet this peculiarity has no effect upon the characteristic impermeability of the cap layer, except in a definite region near the hilum, the strophiole.

Small permeable area at strophiole

All soft seeds which have not been scarified or sulphuric-acid softened have a small permeable area which shows as a black mark with osmic acid staining. It ranges from a short line to a circular area of varying size, and is found centered in a narrow longitudinal depression, close to the hilum on the side opposite the micropyle. The small elevations on either side are usually more highly developed in other leguminous seed, and form the strophiole. Figures 10 and 11 show seeds which have been blackened at the strophiole. This area, even when large, is not conspicuous on a seed lying in a normal position, but it becomes prominent when the hilum is turned uppermost.

When this area is examined under intense oblique illumination the arrangement of the Malpighian cells is striking. At the bottom of each depression certain of these cells show a linear arrangement of their middle lamellae in plane of the center of the hilum, while around these, little or no order is apparent. In soft seeds, staining always occurs about these linearly arranged cells. Photomicrographs taken with oblique illumination are shown in figures 12 and 13. This blackened strophiolar area is found on the mature soft seeds from hand-picked and hulled samples of widely varying ages, and in addition on all unscarified soft seed in commercial samples.

Natural softening occurring many years after the seed matures is of the same nature, as was shown on testing samples 20 years or more in age with osmic acid. These seeds were from sweet clover and alfalfa sample bottles sent out in 1911 by the Dominion of Canada Department of Agriculture, and those that had softened with age had the permeable region at the strophiole. Machine-threshed seeds show a high percentage stained at this point, and a low percentage softened by scarification.

Seeds softened by scarification are permeable to an extent determined by the severity of the operation, and in many cases also through the strophiolar area. Strangely the softness of scarified seeds may be largely of this latter type; and in the Eddy machine, where the seeds are thrown along a carborundum surface, it is mostly of this kind. When hard seeds from hand-picked and hulled samples are softened by heating, this is the area that becomes permeable. This is true of those heated at temperatures below 100° C., either dry or in water. It is these temperatures below the boiling point that other workers have found useful for softening purposes, and investigation indicates that this method produces soft seeds which are indistinguishable from those occurring naturally. While heating has been recommended for softening on a large scale, little use has been made of this method, which may considerably decrease the total germination of a sample unless applied with proper care.

When hand-picked and hulled samples of *Melilotus officinalis*, and of other clovers and alfalfas, were treated with osmic acid, it was clearly evident that softness was invariably due, as in the case of *M. alba*, to the formation of a permeable area at the strophiole. The species so investigated were *M. officinalis*, *Trifolium agrarium*, *T. arvense*, *T. hybridum*, *T. pratense*, *Medicago hispida*, *M. lupulina*, *and M. sativa*.

Discussion: mechanism of softening

The mechanism of natural softening was made clear by a histological investigation of the region in which it occurs. Figure 15 shows a photomicrograph of a section through this blackened area in a permeable seed of *M. alba*. The seed coat is bilaterally symmetrical about a plane extending through the center of the area and the

hilum. On each side of this plane, the Malpighian cells are longer and narrower than normally is the case. On both sides of this, shorter Malpighian cells are found overlying loosely arranged cells, which show occasional linear arrangement. The distribution of the blackened lumina of the Malpighian cells is clearly shown, and it will be noticed that the cells have pulled apart, forming a split along the plane of symmetry. This split is characteristic of all sections that have stained at the strophiole, and it is without doubt the aperture by which liquids gain entrance to soft seeds. The curious anatomy of this region in legume seeds other than sweet clovers, clovers, and alfalfas has been well illustrated by MATTIROLO and BUSCALIONI (**19**).

The initial swelling of an osmic-acid stained seed occurs at the small stained area. It is evident that water enters through the crack and is imbibed by the tissues; pressure develops, and finally swelling occurs when this passes the elastic limit of the coat.

On examination of serial sections cut from a pod containing an immature seed, some additional evidence was found. Figure 16 shows a section through the strophiole. The point of dehiscence of the pod is immediately above, and the raphe, as identified by YOUNG (**33**), is directly below this region. It is evident that the raphe ends in this spot, so that chalazal tissue lies internal to it. Figure 17 shows that the long Malpighian cells in the center have formed caps; those immediately on either side are dividing; and those still farther away resemble embryonic epidermal cells. The long slender Malpighian cells have matured much earlier than those on either side, which have originated like the underlying loose tissue from the Malpighian mother cells.

Figure 14 shows a section through a seed which has remained hard following treatment with hydrochloric acid and ammonia and the subsequent maceration which removed most of the cuticle and subcuticular layer. In the center of this thick unstained section (about 15 μ) the long slender Malpighian cells are distinctly swollen in appearance, due either to actual swelling or to lateral expansion from seed coat tension, and there is no sign of separation, although bowing has taken place. Any section to show this condition must be made with great care, and must be of this thickness at least, otherwise the

Malpighian cells split apart during the process, as they do in the softening of a seed.

The strained condition seen in this photomicrograph is not evident in figure 15, where the splitting has occurred and the tension released. Here the cells are only slightly bent, since lateral displacement of the lower ends has occurred. At their apices the caps and the heavy upper walls have moved just enough to indicate that a crack has developed in the permeable layer of the seed coat.

This evidence, the softening at the strophiole produced by moderate heating and the abundant occurrence of unscarified soft seeds in commercial samples, leads to the view that these strophiolar cells in hard seeds are in a state of metastable equilibrium which, when upset, produces softening through a split along the middle lamellae, linearly arranged in the plane of symmetry. Possibly the more rapid development of these cells has played a part in their ready separation at this point, through a reduction in the adhesiveness of the middle lamellae.

It is believed that this condition of metastable equilibrium explains the presence of soft unscarified seeds in commercial seed samples, which have been subjected to so much throwing about in threshers and scarifiers. In view of this, it was thought probable that fracturing would occur with even a small impact in the vicinity of the strophiole.

To test this hypothesis it did not seem possible to tap seeds individually, but it was easy to shake a number of them in a bottle. Table II gives the results obtained when the seeds were hand-shaken vertically about three times per second in a 500 cc. corked Florence flask.

In many shaking and mechanical impacting experiments, the softened seeds were tested with osmic acid, and invariably the strophiole was blackened. Since blackening elsewhere occurred only at the hilum, except in those rare cases where scarification took place, water must enter these mechanically softened seeds in the same way that it does in those in which softening has occurred by natural processes. It may be noted that the osmic acid test is expensive when employed on a large scale. Under such conditions also, care must be taken to protect the users from the poisonous vapor.

That severity of impact is necessary for seed softening is certainly not indicated by these results, nor in the experiments mentioned later in which mechanical impacting was employed. It is important, however, that enough impacts should occur so that at least one blow will strike at, or near, the strophiole. Although the severity of the impact is small when the seed is shaken in a flask, yet the number of impacts per seed is rather high when the sample is almost completely softened. In the experiment summarized in table II, the maximum number of impacts per seed, calculating that each seed would strike the bottom once and the cork once per shake, would be $10 \times 60 \times 3 \times 2$, that is, about 3600 effective times, or half that number if it is assumed that the cork was too soft and the sides at the

TABLE II

HAND-HULLED SEEDS OF M. ALBA SHAKEN FOR 10 MINUTES AT THREE OSCILLATIONS PER SECOND IN A 500 CC. CORKED FLORENCE FLASK*

SEEDS	NUMBER OF SEEDS	NUMBER REMAINING HARD	NUMBER PERMEABLE	PERCENTAGE PERMEABLE
Control (unshaken)	884	880	4	0.5
After shaking	901	71	830	91.0

* Seeds were tested for permeability by soaking for two days in water at room temperature.

top too sloping to be considered effective agents. These figures indicate that either a large number of small impacts are required for softening, which seems rather incredible, or that softening occurs only on the seed striking in one particular spot.

Both stained and unstained softened seeds have been stored for weeks in a dry place and have promptly absorbed water upon soaking. This fact, together with the evidence that the softening occurs through fracturing, indicates that ESDORN'S (10) suggestions that hardness is a reversible state and that permeability occurs over the whole surface of the seed have no bearing on the phenomena in *M. alba* or in its related species.

Many other shaking experiments have been performed and have given efficient softening, although this method is slow and awkward. To eliminate the uncertainties of shaking, a number of impactors were designed which differed in detail rather than in the centrifugal principle employed. In these machines, the seeds were dropped on a

radially flanged revolving plate, and were centrifugally thrown against a peripheral vertically ribbed wall. In each machine the revolutions per minute and the kind of flanging on the disk and ribbing on the wall differed in some respect, as did the efficiency per operation.

The tabulations of the experimental results show approximately the same percentage softening of the hard seed content per operation for any one machine. Because of this, unless there is very efficient softening per operation, many operations would be required in some cases completely to soften a sample. For example, if the sample initially contains 100 per cent hard seeds which are passed through a machine whose efficiency of softening per operation is 50 per cent, then the stages of this theoretical experiment would show the following results:

Stage	1st	2nd	3rd	4th	5th	6th	7th	8th
Percentage hard seeds present	100	50	25	12.5	6	3	1.5	0.75

Thus when the hard seed content of the sample was reduced to two seeds, one more operation would reduce it to one seed, and two more would be required to soften the remaining seed. This theoretical example demonstrates the relationship between successive operations in the succeeding mechanical experiments.

In tables III, IV, V, and VI are shown the results secured with impactors 2a, 2b, 2c, and 2d, where the severity of impacting was thought to be approximately the same. This was secured by running the disks at the same peripheral speed, although there was a variation introduced by the differently corrugated walls.

The procedure in these experiments was as follows. Sufficient hand-picked seeds were hulled and cleaned, and these were then divided into approximately equal parts, one for the control and one for each separate stage of the experiment. Following the treatment, the experimental lots and the control were placed in water for two days. At the end of this period all seeds were counted, except the few immature seeds and those with hulls which had not been removed during the cleaning process. It is believed that the presence of these during the experiment did not significantly affect the results.

In the first of these experiments, performed with impactor 2a, the seeds were discharged by a 4-flanged disk against a smooth tin wall. A real impact would occur when the seeds were struck by the revolving flanges. Table III gives the results.

TABLE III

Number of times sample passed through impactor 2a	Number of seeds	Number remaining hard after 2 days' soaking	Number swelling	Percentage soft
Control	1070	1065	5	0.5
1	660	627	33	5.0
2	619	570	49	8.0
4	656	536	120	18.0
8	1135	782	353	31.0
16	752	533	219	29.0*

* This is a curious result which as yet has not been explained.

In the next experiment, impactor 2b was used. It differed from the preceding by its slightly smaller disk and corrugated peripheral wall. A larger number of impacts took place on this wall, and gave higher efficiency. The results are given in table IV.

TABLE IV

Number of times sample passed through impactor 2b	Number of seeds	Number remaining hard after 2 days' soaking	Number swelling	Percentage soft
Control	689	685	4	0.6
1	784	520	264	34.0
2	937	465	472	51.0
4	540	136	404	75.0

Mechanical impactor 2b was the previous impactor 2a modified so that the striking surface gave an increased number of minor knocks.

Impactor 2b did not treat the seeds in a uniform and steady manner, so impactor 2c was devised. In this machine the corrugated wall was more deeply crimped, the disk was decreased in diameter to allow the seeds to move more freely, and eight additional flanges were added. The increase in the number of driving surfaces probably caused the 15 per cent greater efficiency in softening. The experimental results are given in table V.

It was believed that the coarse crimping and roughly spinning disk in impactor 2c prevented higher efficiency in softening. This was obtained when the wall was very slightly though finely corrugated, and an aluminium disk of the same dimensions was substituted for that used in impactor 2c. Particular care was taken in smoothing edges and rounding corners of the latter. The results obtained are shown in table VI.

TABLE V

Number of times sample passed through impactor 2c	Number of seeds	Number remaining hard after 2 days' soaking	Number swelling	Percentage soft
Control	870	866	4	0.5
1	954	488	466	49.0
2	801	246	555	69.0
4	1029	120	909	88.0

Mechanical impactor 2c was the previous impactor modified so that an increased number of effective driving surfaces were secured.

TABLE VI

Number of times sample passed through impactor 2d	Number of seeds	Number remaining hard after 2 days' soaking	Number swelling	Percentage soft
Control	426	424	2	0.5
1	284	109	175	62.0
2	801	117	684	85.0
4	667	26	641	96.0

Both the striking and driving surfaces were further changed in this machine, and in addition the channels were so modified that the seeds passed through more smoothly.

A final series of experiments was performed with machines which gave two severe blows per operation, one when the seed was driven and the other when it was stopped. In these machines vertical ribs were soldered to the peripheral wall at right angles to the driven seeds. A 4-flanged disk drove the seeds against four ribs in impactor 3a, and the results are shown in table VII. The low efficiency of this machine shows that either severe impacts alone do not cause softening, or else the seeds passed through without being struck. To avoid the latter possibility, impactor 3b was made with twelve flanges on

the disk and twelve ribs on the wall. These were placed slightly off the vertical, to prevent seeds bouncing back against the disk. The results are shown in table VIII. This machine showed slightly lower efficiency than the previous one, but the noise indicated that prob-

TABLE VII

Number of times sample passed through impactor 3a	Number of seeds	Number remaining hard after 2 days' soaking	Number swelling	Percentage soft
Control	631	630	1	0.2
1	845	636	209	25.0
2	713	421	292	41.0
4	756	217	439	58.0

The striking surface of this machine was designed suddenly to stop the motion of the driven seeds, and so give severe impacts.

ably every seed was impacted, and severity was shown by a maximum of 15 per cent scarification. To obtain lower scarification the machine was run at approximately half the speed, and the results are shown in table IX.

TABLE VIII

Number of times sample passed through impactor 3b	Number of seeds	Number remaining hard after 2 days' soaking	Number swelling	Percentage soft
Control	621	617	4	0.6
1	418	325	93	23.0
2	461	304	157	34.0
4	691	249	342	49.0

The striking surface of this machine was of the same kind as impactor 3a, but modified so that a larger number of seeds would receive the same severe treatment. Up to 15 per cent of the seeds in this experiment were scarified, probably when the rapidly moving seeds struck the sharp corners.

These experiments, both hand and mechanical, demonstrate that softening is effectively carried out by light impacting. Heavy impacts are no more effective and are associated with scarification. While many blows are required to ensure softening of the whole sample, the experiments indicate that softening is not a cumulative effect. On the other hand, the implication is that a large number of blows merely increases the chances of a single blow striking the stroph-

iole. When this occurs with sufficient force to upset the stability of the tissue, which is in a state of tension, then a cleft appears on the separation of the long slender Malpighian cells, permitting the passage of water through the now permeable seed coat.

TABLE IX

NUMBER OF TIMES SAMPLE PASSED THROUGH IMPACTOR 3b	NUMBER OF SEEDS	NUMBER REMAINING HARD AFTER 2 DAYS' SOAKING	NUMBER SWELLING	PERCENTAGE SOFT
Control	157	148	9	5.7
1	196	172	24	12.0
10	204	169	35	17.0
20	289	219	70	24.0

Half-speed operation reduced scarification to nearly zero, and efficiency was greatly reduced per operation.

When these methods of hand and mechanical softening were extended to other species, a limited amount of experimental work indicated that softening of the clovers and alfalfas mentioned may also be accomplished by impacting as has been done in *M. alba*.

Summary

1. The impermeable region of the seed coat of *Melilotus alba* is formed by a layer of tightly appressed suberin caps.

2. Hard and soft seeds can be distinguished in the unswollen condition by the use of osmic acid.

3. Permeability of naturally soft seeds occurs through the opening of a cleft at the strophiole. This strophiolar cleft can be produced by moderate heating or by mechanical impacting.

4. Soft-seededness is an irreversible condition.

5. A limited amount of work with several other legume seeds has indicated the presence of similar characteristics.

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EXPLANATION OF PLATES IV, V

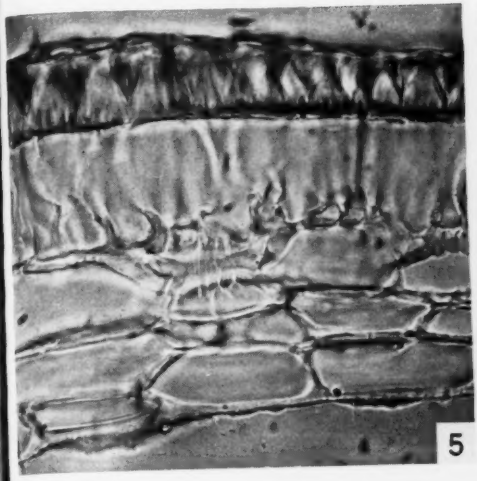
All illustrations are of *Melilotus alba*.

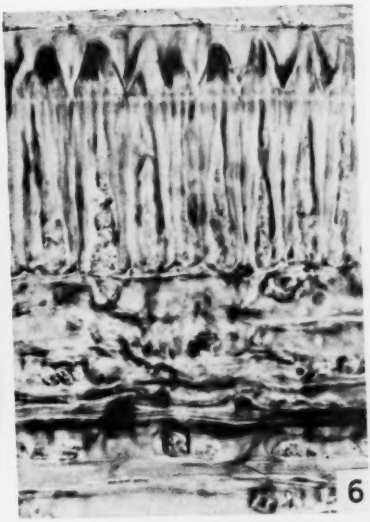
FIG. 5.—Radial section of seed coat showing effect of copper oxide ammonia treatment; unstained; ×992.

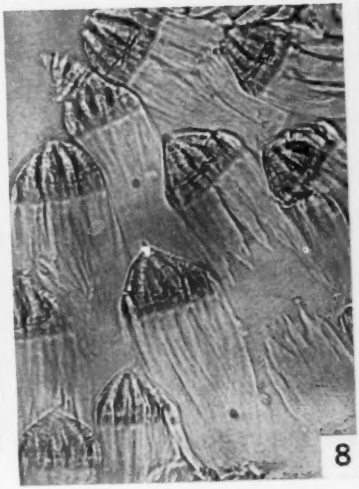
FIG. 6.—Radial section of seed coat; stained with ruthenium red; ×1020.

FIG. 7.—Part of Malpighian cell remaining after maceration with hydrochloric acid and ammonia; unstained; ×867.

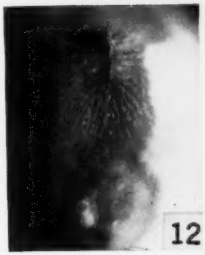
FIG. 8.—Seed coat macerated with chromic acid, showing caps and part of lower walls of Malpighian cells; unstained; ×867.

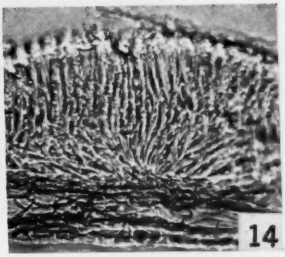


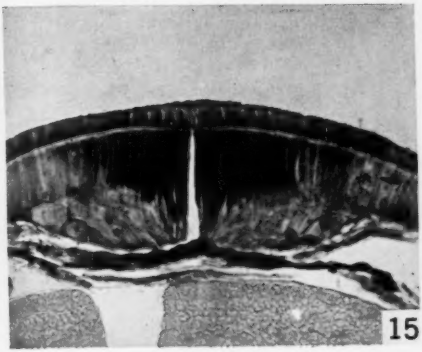


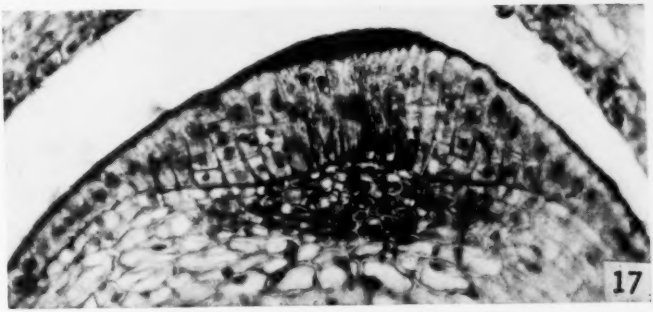


HAMLY on MELILOTUS









HAMLY on MELILOTUS

FIG. 9.—Caps of Malpighian cells remaining after incomplete dissolution of seed coat in chromic acid; unstained; ×867.

FIG. 10.—Seeds mounted to show hilum and strophiole: hard seed on left, soft seeds on right (stained with osmic acid); ×6.

FIG. 11.—Seeds in normal positions: hard seed on left, soft seeds on right (stained with osmic acid); ×6.

FIG. 12.—Superficial view of hard seed at strophiole; oblique illumination; ×42.

FIG. 13.—Superficial view of seed softened by shaking; osmic acid has stained some strophiolar tissue; oblique illumination; ×42.

FIG. 14.—Unstained transverse section of strophiole of seed remaining hard after treatment with hydrochloric acid and ammonia; ×210.

FIG. 15.—Transverse section through strophiole of an osmic acid-stained soft seed, showing elongated blackened Malpighian cells and cleft through which liquid entered; ×345.

FIG. 16.—Section across strophiole of immature seed within its pod; ×134.

FIG. 17.—Transverse section of strophiole of immature seed, showing developed and undeveloped state of Malpighian cells; ×346.

IRREGULARITIES IN THE RATE OF ABSORPTION BY DRY PLANT TISSUES[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 435

CHARLES A. SHULL AND S. P. SHULL

(WITH THIRTEEN FIGURES)

Introduction

In previous papers the writers reported some very careful studies on the absorption of water by dry colloidal materials of plant origin. The purpose of those studies was to test the water-simplification theory of absorption put forward by BROWN and WORLEY (1), and, by analysis of the data mathematically, to determine the type of curve which fitted most accurately to absorption data. As a result of the studies, certain features of the BROWN and WORLEY theory were rejected, and a new theory was proposed which was more nearly in harmony with the facts. The work was done on various substances, such as pea cotyledons, seeds of cockleburs (7), seeds of *Zea mays* (9), and gelatin (8).

Under ideal conditions, that is, constant temperature, accurate timing, uniform and rapid drying technique, physical and chemical homogeneity of material, and chemical stability of the absorbing substance in the presence of water at room temperature, the imbibition occurs with a continually diminishing speed. The rate of absorption varies from moment to moment according to definite laws. The rate of intake is high at first, but falls off rapidly with each increment of moisture, due to the increasing satisfaction of the forces which cause hydration. As saturation is approached, the rate of absorption approaches zero and finally ceases, or is balanced by the outward diffusion of soluble substances from the tissues.

The changes in rate are so typically regular under these ideal conditions that mathematical expressions can be developed which will follow the data for considerable distances along the curves, with

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

astonishing precision. The formulae fitting the data most closely are logarithmic in type, and may be represented in a generalized expression by the following equation: $y = a \cdot \log_{10}(bx+1)+c$, in which y is the percentage of water already absorbed, and x is the time in minutes since absorption began. The symbols a, b, and c are constants. Every case of *uniform* absorption so far studied can be followed by curves of this type, merely by choosing appropriate values for the constants.

The *rate* of water entry at any given moment can also be represented by the equation $v = ae^{-k\phi}$, in which ϕ is the percentage of absorption that has already taken place. This formula means that at any given moment the rate of water intake is an inverse exponential function of the amount of water previously absorbed. In other words, the rate of imbibition depends upon the amount of water already in the tissue, or upon the degree of saturation deficit prevailing in it at the moment the rate is measured. The less saturated the hydratable colloids are, the greater is the rate of absorption in the presence of free water; and the more fully hydrated they are, the slower is the rate of absorption. These statements are true, whether the water enters as liquid in direct contact with the tissues or as a gas from a saturated atmosphere. These relations are in harmony with everything else we know regarding the relations of organic gels to water.

During the studies we found many specimens, and many kinds of material, that exhibited irregularities in absorption rates. Indeed, it is commoner to find the absorption rates irregular than regular. In several cases we suggested causes for the irregularities observed (**6, 7, 9**). In this paper we are stressing the irregularities, with particular reference to the significance of the observed changes in the rate of water intake. Such a discussion is perhaps desirable, since a case of irregularity in absorption by the seeds of the Indian lotus has been described by OHGA (**5**), and, we believe, misinterpreted.

The data from which we have drawn for this presentation have been accumulated over a period of years. Part of the work was done some years ago at West Virginia University, part of it at the Oregon State Agricultural College, and part of it at the University of Chicago. Acknowledgments and thanks are accorded to Dr. P. D.

STRAUSBAUGH and Dr. R. B. DUSTMAN of West Virginia University, to Dr. H. P. BARSS and Dr. W. M. ATWOOD of the Oregon State College, and to the administrative authorities of these institutions for many courtesies and laboratory privileges. We are also indebted to Mr. JOHN W. MITCHELL for faithful assistance at Chicago.

Materials and methods

The methods used in all of the work are identical with those used in the previously cited papers. The utmost care has been taken to minimize the inherently unavoidable errors. A great variety of material has been used. Thus in the studies of absorption by pea cotyledons we used the Alaska Bush pea, Canada Field pea (both yellow and green), Small Scotch yellow, Tom Thumb, and Champion of England pole pea. It is not necessary to present all of the material here, as the results are similar for all of them. As the Canada Field pea has hard round starchy cotyledons, and the Champion of England pole pea has wrinkled cotyledons of the sugary type, these two have been chosen for presentation. The Champion of England pea was heterozygous for color, but was selected, only green cotyledons being used.

Among the varieties of corn used were Boone County White, Silver Mine, Hickory King, McKay Yellow Dent, and Reid's Yellow Dent. The Silver Mine variety has been chosen as giving typical behavior, and is the only one presented. Other data were obtained from *Xanthium italicum*, seeds of *Hibiscus* (rose mallow), *Gossypium*, and from the fruiting body of the fungus *Auricularia auricula-judae*.[2]

These materials have been selected because they illustrate a number of the most important types of irregular absorption behavior. As the absorption data in these cases are not susceptible of mathematical analysis, nothing new can be added to the mathematical discussion presented in previous papers. For this reason also, it has seemed better to present the data in the form of graphs, rather than as tabulated material. It would be difficult to see the irregularities

[2] The specimens of *Auricularia* used were furnished by the late Dr. C. H. KAUFFMAN, of the University of Michigan, and by Dr. WM. A. ANDERSON, Jr., of the University of Iowa.

from the tabulated figures, and there is not sufficient justification for use of both tables and graphs in this case.

Results

A. Formation of internal cavities

A relatively common cause of irregularities in the rate of water intake in the case of seeds and naked cotyledons is the formation of internal cavities during imbibition. This has been observed in all

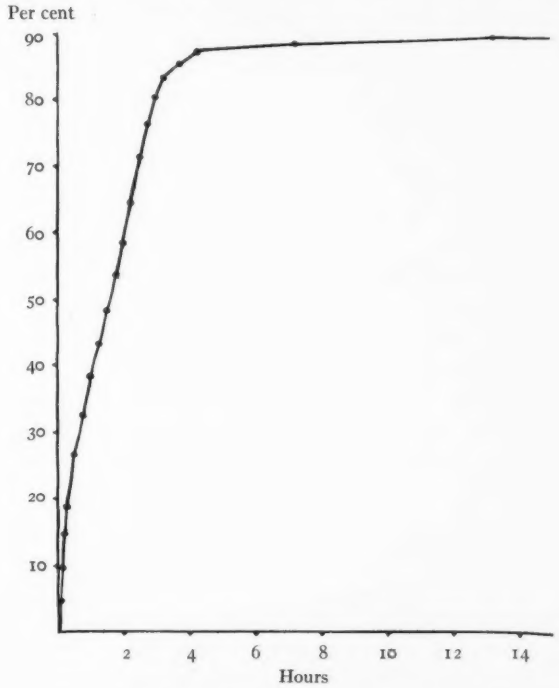


Fig. 1.—Curve of total intake of water for starchy cotyledons of Canada Field pea (yellow) at 23° C.

varieties of peas tested, and also in all of the types of corn grains used. Figure 1 is derived from data obtained with cotyledons of the yellow-seeded Canada Field pea, soaking in water at 23° C.; and

figure 2 from cotyledons of the wrinkled green cotyledons of the Champion of England pole pea at 26° C. The upward swing of the curves can be seen most clearly by placing the eye at the point of origin, and sighting along the curve. The secondary increase in absorption rate usually occurs during the first or second hour, with intake of 50–70 per cent in the Canada Field pea, and with 60–100

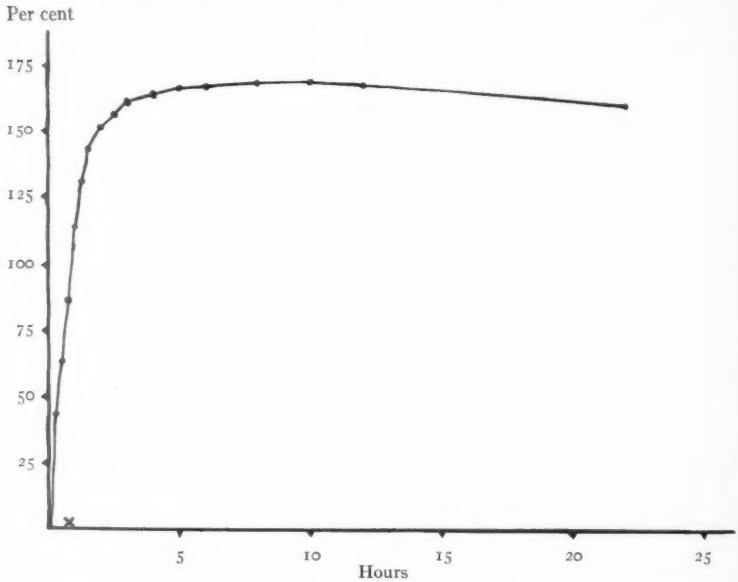


FIG. 2.—Curve of total water intake for green sugary cotyledons of Champion of England pole pea at 26° C.

per cent in the Champion of England pole variety. Figure 3 represents the irregularity in a different way. The increase in weight in each period of absorption is expressed in milligrams per minute. This method does not actually plot the rate of absorption, for to do that properly the time intervals should be made infinitesimal, as is pointed out later. Nevertheless it does reveal the character of the rate curve, and the data as presented could be obtained without attempting an impossible mathematical analysis. The longest line at the left of figure 3 measures the total intake (not the rate) during

the first minute; the second line the intake during the second minute; and the third line is the average intake for each minute during the third, fourth, and fifth minutes of absorption. Then follow lines representing the averages for the sixth to tenth, eleventh to fifteenth, and sixteenth to thirtieth minute. During the second half-hour there is actually a greater average intake per minute. The values for intake from the thirty-first to forty-fifth, and from the forty-sixth to sixtieth minutes are indicated by *x* in figure 3. Following this abnormal period of intake, the average per minute intake falls to normal during the first quarter of the second hour; and from that on to the end at 10 hours there are no other serious irregularities. The last line at the right (eighteenth period) represents average-per-minute intake for the ninth and tenth hours, during which period the intake was only 0.0075 gm., or 0.0000625 gm. per minute.

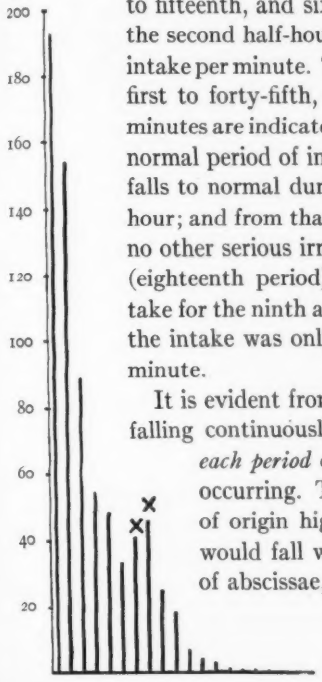


FIG. 3.—Graphic representation of the approximately relative gain in weight per minute (mg.) during successive periods of absorption; Champion of England pole pea, green cotyledons, at 26° C.

It is evident from the graph that the *rate* of intake is falling continuously, and it falls continuously during *each period* except when an abnormal increase is occurring. The *rate* curve would have its point of origin high up on the axis of ordinates, and would fall with decreasing steepness to the axis of abscissae, with lapse of time. If there is any irregularity it will appear as a hump on the rate curve if the irregularity is an abnormal increase in rate, or as a trough in the curve if there is a temporary abnormal decrease in rate.

When the absorption occurs in a solution of some substance that increases the rate of absorption, and especially the total amount of absorption, the irregularities are often more pronounced. This situation is illustrated in figure 4, which shows the curves of absorption for the cotyledons of the Canada Field pea (yellow) in solutions of sodium hydroxide. The lower curve in figure 4 represents the ab-

sorption in 0.1 molar NaOH, and the upper curve is from data obtained with 1.0 molar solution. The temperature was 23°–24.5° C. during the test. In these cases the starch and other polysaccharides were gelatinized by the strong hydroxide solutions. The swelling of the superficial portions of the cotyledons is not only hastened but much increased, so that the strains set up in the tissues are much greater. On account of the chemical effects of the alkali upon the cell walls and starch, the weighings could not be continued with accuracy beyond 11 hours.

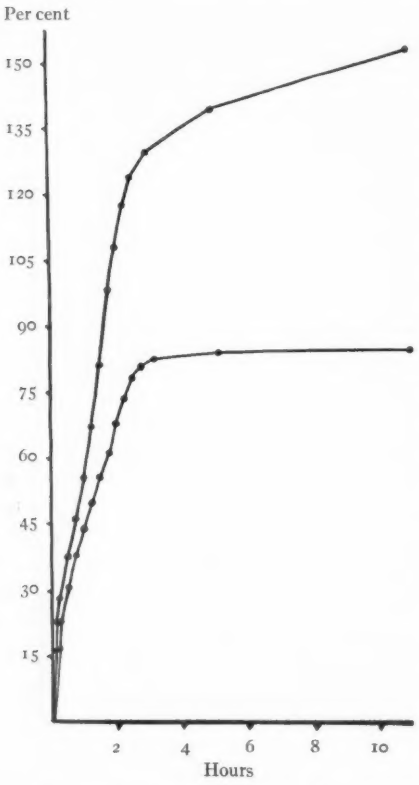


FIG. 4.—Curve of total absorption of cotyledons of Canada Field pea (yellow) in NaOH: upper curve, 1.0 molar; lower curve, 0.1 molar solution.

Following the periods of abnormal intake, the cotyledons were examined for internal cavities. On cutting the cotyledons in directions parallel and perpendicular to the flat side of the cotyledon, it was easy to observe the cavities produced during swelling. They were sometimes crescent-shaped, sometimes stellate or irregular, the breaks apparently following lines of least resistance through the tissues. About 80 per cent of the cotyledons showed these cavities after absorption was completed. The behavior and appearance of the cotyledons during absorption have been observed with minute care, and from changes in shape

which occur during the secondary rise in the curve of total absorption it is confidently believed that the formation of the internal cavities coincides with the period of secondary increase in absorption. Apparently the early swelling of the superficial portions of the cotyledons produces a tension which breaks the dry interior un-

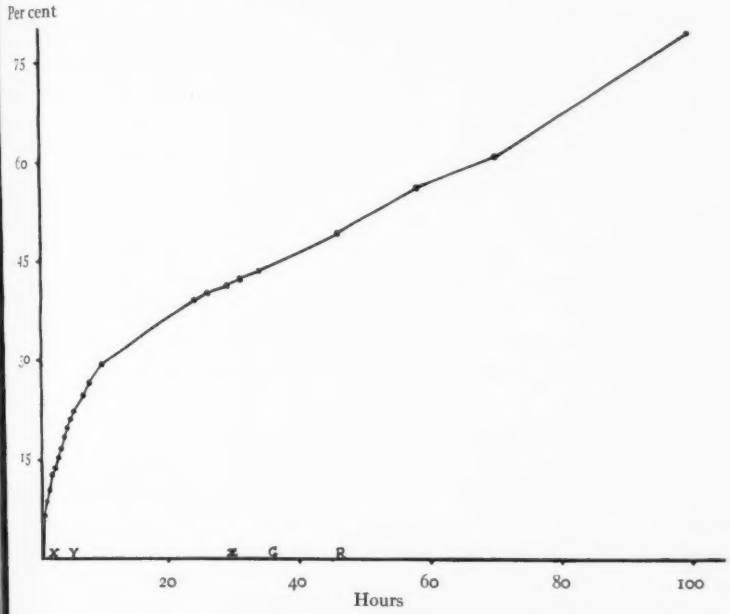


FIG. 5.—Curve of total absorption of Silver Mine corn, 22° C.: irregularities at *x*, *y*, *z*; germination at *G*; root absorption by osmosis at *R* and beyond.

swollen portion. Into the cavity thus formed water is drawn, not by true absorption, but by the development of an internal negative pressure. The curves of figure 4, with NaOH solutions, should be compared with figures 1 and 2, with water. The irregularities are greatly exaggerated in figure 4.

Similar irregularities are produced during the swelling of corn grains. The curve of imbibition for Silver Mine corn is shown in figure 5. The first irregularities in the weighings were observed at the end of about two hours at point *x* on the curve. Other breaks in

the curve occurred during the sixth hour at *y*. These irregularities were accompanied by the cracking up of the interior of the endosperm as the outer parts swelled. The cracks in the endosperm were plainly visible through the pericarp, and the formation of these cracks was simultaneous with the increased absorption. Much later, at the end of about 30 hours, a third period of irregular absorption occurs, at point *z* on the curve. This period is probably associated with embryo activity on approaching germination. Only a few hours later (36-hour point on curve), at *G*, nine of the ten seeds showed the first protrusion of the radicles through the pericarp walls. These walls are probably stretched by the pressure of the enlarging radicles before the pericarp is actually torn. Such stretching would cause unusual intake at the time of its occurrence.

At this time the seeds were removed from the vessels in which they had been soaking and were arranged on absorbent cotton with abundant moisture, and the subsequent absorption was mainly osmotic. At the close of the experiment (100 hours) all of the seeds had germinated, and the longest radicle was 6 cm. and the shortest 2 mm. in length.

The formation of internal cavities by breaking of the endosperm at *x* and *y* in figure 5 is to be explained on the same grounds as the formation of cavities in the pea cotyledons—expansion of the parts of the grain immediately beneath the pericarp by swelling, while the interior of the endosperm is still dry and unswollen.

B. Osmotic action

It has been observed in several cases that osmotic activity of the seed coats is responsible for irregularities in absorption behavior. Under ordinary conditions it will not be observed with absorption from pure water; but when certain acids (such as acetic) or alkalies (NaOH, KOH) are used, semipermeable testas may greatly modify the course of weight increase. It may be questioned, of course, whether the phenomena observed in such cases should be called absorption, for there is usually much free liquid inside the seed coat. It is held merely by osmotic action, and not by the seed substance. The most striking results have been obtained with the seeds of various species of *Xanthium*. Our experiments have usually in-

volved the seeds of *X. italicum*. When soaked in water these seeds absorb at a uniformly decreasing rate with time. There may be no irregularities at all, since there seem to be no cracks nor breaks

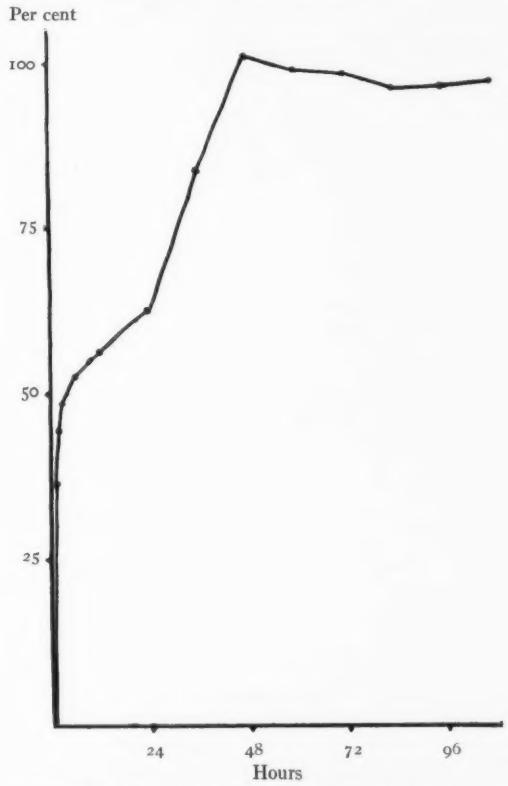


FIG. 6.—Curve of total absorption of *X. italicum* seeds in molar solution of NaOH

developed internally during the process. However, when acids or bases are used the rate of absorption is greatly increased, provided the testas are not impervious to these reagents. In figures 6 and 7 are presented the results of two experiments with *X. italicum* seeds which were soaked in molar NaOH solutions. When pure water is used, saturation is usually accomplished with about 52 per

cent increase in weight. In figure 7 the approximate position of the saturation curve with water is indicated by the broken line, while the heavy line above the junction of the two curves shows the increased absorption or intake due to osmotic action. Figure 6 shows the secondary rise of the curve somewhat better, because the effects of the osmotic activity were delayed slightly longer in appearing.

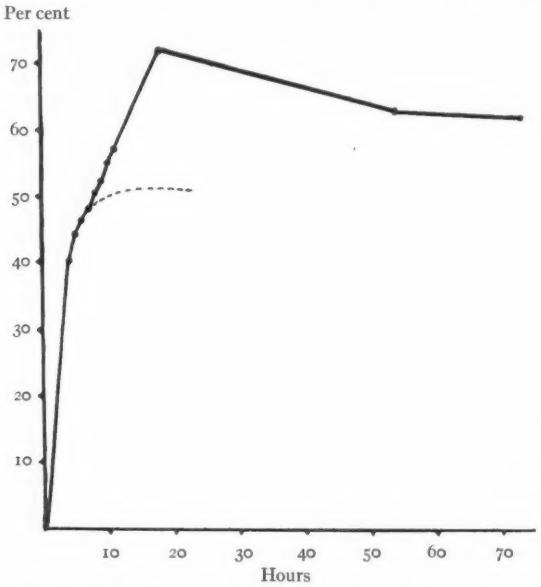


FIG. 7.—Curve of total absorption of *X. italicum* seeds in molar solution of NaOH; usual point of saturation in water indicated by broken line.

At the time the maximum absorption is registered in these cases, the seeds are inordinately swollen. The seed coats are stretched away from the embryos, and in many cases (about 90 per cent) the outer layers of the seed coats burst from the excessive intake of liquid.

The explanation of the irregularities in this case is that after the first period of regular absorption has occurred, up to approaching saturation of the seed substance, the acid (acetic) or alkali (NaOH or KOH) which has penetrated the coats in time causes hydrolysis of

the food reserves. As a result of this action, a concentrated solution of organic solutes is developed inside the seed coats. Since these testas are practically impervious to organic solutes, the conditions for high osmotic pressure are rapidly developed. Water quickly enters with the stretching action of the pressure, giving rise to the secondary period of absorption, and to the bursting of the less yielding outer layers of the coats.

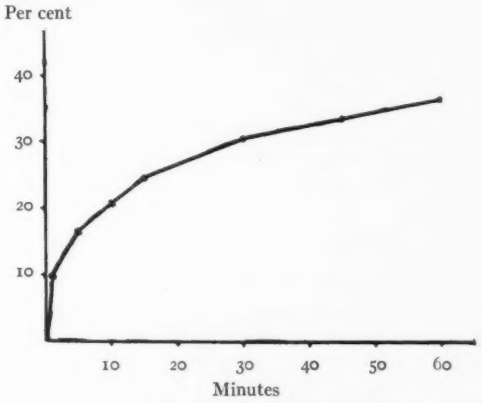


FIG. 8.—Curve of total absorption of *X. italicum* seeds during the first hour in molar NaOH solution.

In order to show that the *rate* of intake falls off with time even in the steepest parts of these curves, the first hour of absorption in figure 6 has been redrawn to a different scale in figure 8. The increase in weight during this period was 36.72 per cent. By inspection of the figure it is obvious that the rate of intake is much greater during the first moments of absorption than at any later period.

Previous studies have shown (**6**) that a number of chemical substances will penetrate the coats of *Xanthium* seeds, and that other substances such as NaCl and sugar (**6, 2**) do not noticeably penetrate. Any substance which can penetrate the coats, and which after entrance effects the decomposition of the organic constituents of the embryo and its reserves, will produce the changes in absorption rates here described.

C. Non-homogeneity of material

A third cause of deviation from regular absorption rates is lack of physical homogeneity in the absorbing material. In many kinds of seeds the outer parts of the seed coats may be heavily cutinized and impervious to water. Sometimes the seeds resist the entry of water for weeks (*Sphaeralcea remota*, *Canna*, *Gymnocladus dioica*, etc.), and sometimes resistance persists for only a few hours or a few days (*Hibiscus*, *Gleditsia*, and other legumes). The seeds of various rose mallows have yielded very interesting results. The curves of absorption for several seeds that resisted entrance of water for different lengths of time are shown in figure 9. The first curve at the left is of a seed which was not very resistant. The intake of water was only about 2 per cent in the first two hours; but at the end of five hours over 70 per cent of its dry weight had been absorbed.

The middle curve in figure 9 records data for a seed that resisted the entry of water until near the end of the first day, after which it imbibed rapidly. The third curve at the right in this figure gives the results for a seed that remained in the water for 35 hours before water intake commenced. The variability and irregularity of the behavior is due to differences in degree of waterproofing the seeds. Once the outer layer becomes softened, the remainder of the seed, with its high hydration capacity, exhibits remarkable absorptive power. In other work with the Crimson Eye rose mallow, seeds weighing on the average 0.00994 gm. each were kept continuously in water for 30 days, except during occasional weighings. At the end of this long period of soaking they were found to weigh on the average 0.0099 gm. per seed. In this case the seeds were completely impervious to water during an entire month. While we have no evidence of secondary periods of absorption in this case, it is nevertheless a type of irregularity with which we must reckon in studying the absorption rates of all hard-seeded species. One could easily obtain curves showing secondary periods of increased absorption, if the work were being done with numbers of seeds some of which were resistant and admitted water only after the majority had become nearly saturated.

Another very interesting case of the effects of non-homogeneity on the rate of water absorption has been observed in another member

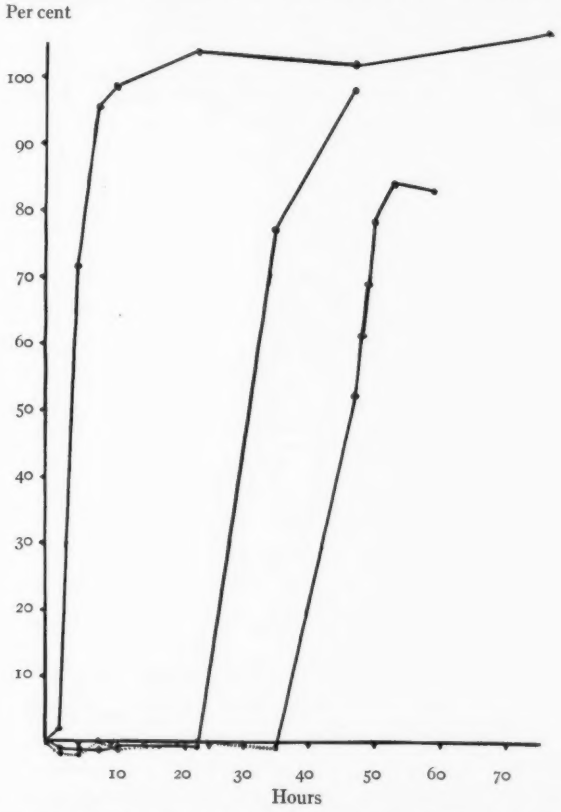


FIG. 9.—Curves showing total intake of water by rose mallow seeds. The start of absorption is delayed for variable periods of time by lack of homogeneity of the material (waterproofing).

of the Malvaceae, cotton. Seeds of cotton were used in three conditions: with the lint left on, with the lint removed by sulphuric acid, and with the heavy brown seed coat removed entirely. The rate of water intake is very different for each of these conditions. Figure 10

has been drawn from data furnished by Miss AUVAL BROWN, who has been investigating the influence of sulphuric acid delinting upon germination, planting operations, and yield of cotton in Oklahoma.

The lowest curve in figure 10 represents the water intake with the lint left on. The seeds were planted on wet absorbent cotton in petri dishes. After the lint is soaked for some time, absorption is more rapid. The middle curve of the figure shows the curve of intake with delinted seeds. The curve is very similar to that of the rose mallow at the left in figure 9. There is a little retardation right at the start, but much more rapid imbibition than in seeds with lint. The retardation would probably be more noticeable if the delinting were done with some agent that did not affect the permeability of the coats.

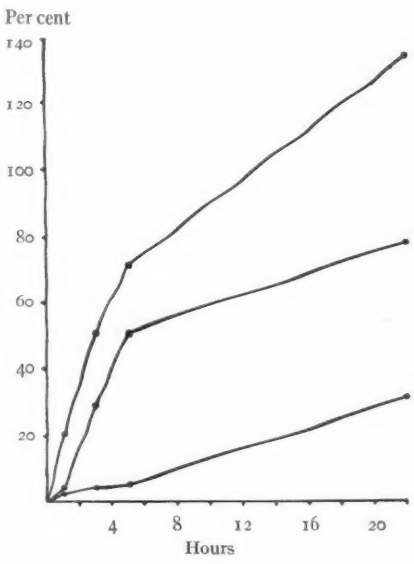


FIG. 10.—Curves of water intake of cotton seeds under different conditions: lower curve, lint attached to seed; middle curve, seed delinted with H_2SO_4; upper curve, seed coats removed completely.

The upper curve in figure 10 is for a seed with the brown coat removed. The embryo and endosperm now have direct access to the water, and absorb 130 per cent of their own weight in 22 hours. It is evident that under ordinary circumstances the rate of water intake here is determined not by the embryo, but by the coat and its appendages. While it is evident that the more resistant parts are on the outside in this case, it is easy to see how a different arrangement of the resistant tissues could produce irregularities of the type that lead to secondary increases in absorption rates. If a more resistant layer lay between two less resistant layers, there would be

a rapid period of absorption, a decline because of the more resistant layer, and another period of rapid intake as this layer softened and admitted water to the second less resistant tissue.

D. Hydrolysis

One of the most peculiar cases of secondary increase in the rate of water absorption has been observed in tissues rich in pectic constituents. The seeds of *Gymnocladus dioica* during germination probably exhibit this type of secondary increase in absorption. But the best material for demonstration of this type of irregularity is the tissue of the jew's ear fungus, *Auricularia auricula-judae*. Figure 11 shows a specimen of this fungus in dry and swollen state. When a piece of it is placed in contact with water, it absorbs very rapidly. During the first five minutes of soaking the *weight* was more than doubled (128.2 per cent increase), but the *volume* increase seemed to be much less than the weight increase. The tissue is very porous, which accounts for the discrepancy between volume and weight change. During the first 24-hour period, with temperature held at 22° C., the weight increase was 452 per cent; but a rapid decrease in the *rate* of absorption is noted in the curve (fig. 12) during the last 21 hours of the first day. During the succeeding four days the rate of absorption is very uniform. This portion of the curve, from the end of the first to the end of the fifth day, is essentially a straight line. The rate was relatively slow, however, for the weight increased only 84 per cent (452 to 536) during 96 hours, a rate less than 1 per cent per hour, as compared with a gain of 346 per cent during the *first half-hour* of absorption.

Beginning with the sixth day of soaking, the curve shows a significant change. The rate of absorption begins to increase again, and becomes more and more rapid with time, reaching a climax at about the ninth day. During this period the tissue that forms the middle portion of the fungus undergoes a change in chemical composition in which it is converted into a translucent gel of extraordinary hydrophilic character. This hydrophilic material gradually dissolves, and the fungus is split into two thin layers.

Microscopic examination of a cross-section of the fruiting body shows that the hyphae in this middle portion run parallel to the

surface. On either side of this middle layer there is a thick layer which is particularly tough near the surface. These tough superficial portions give shape and protection to the deeper parts. The hyphae

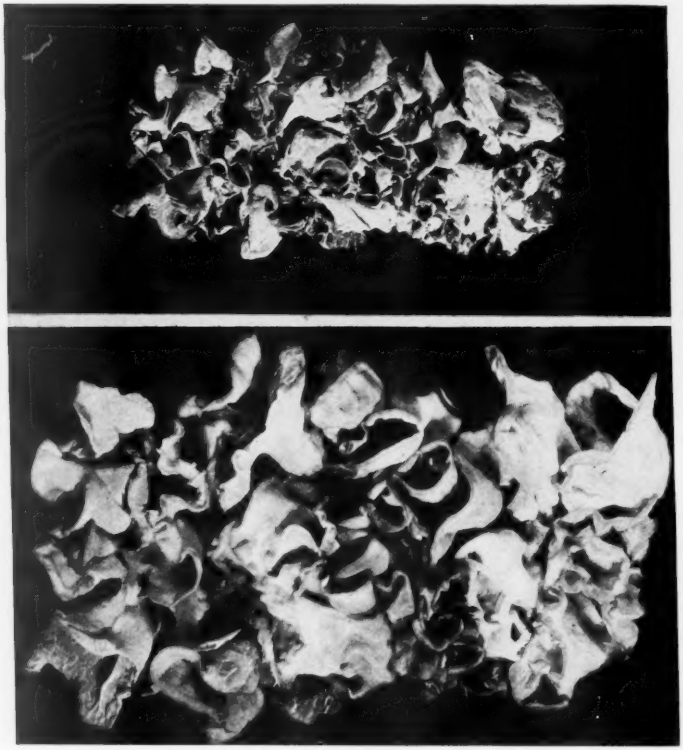


FIG. 11.—*Auricularia auricula-judae*, a fungus; photographs showing fruiting body in dry and swollen state.

are matted and woven together, and run more or less perpendicularly to the surface. The surface itself is covered by a thin layer of loose hyphal ends.

The main swelling of the fungus occurs in the deeper portions of the tough feltlike layers lying on either side of the central conductive

tissue. It is this middle layer which finally breaks down by slow gelatinization and solution during the long contact with free water. When the material has finally separated into two layers, the outer

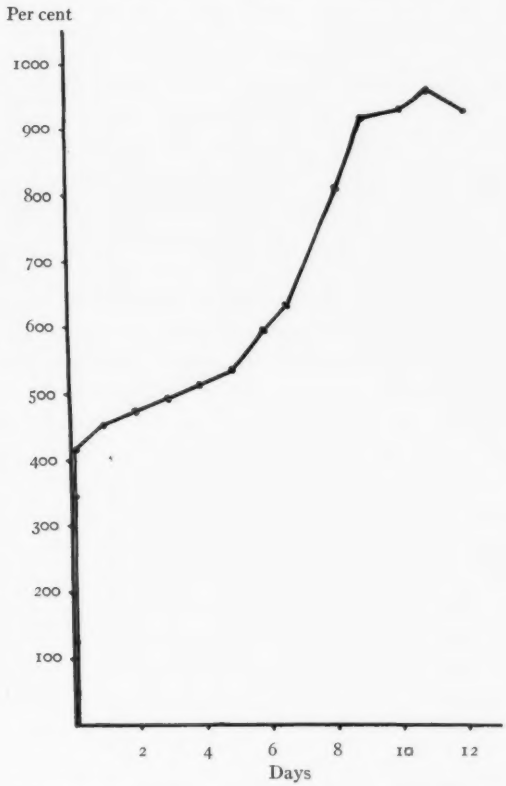


FIG. 12.—Curve of total water intake of tissues of *Auricularia auricula-judae* at 22° C.

surface of each piece is tough and membranous, and the inner surface covered with an opalescent pectic jelly.

After casual examination with ruthenium red had confirmed the belief that pectic substances were involved, a more careful micro-

chemical examination[3] was made. From the results of these tests it appears that the pectic substances are present in the form of insoluble pectic acids, and that after some days of soaking these are converted by hydrolysis into more soluble pectins. The secondary increase in absorption rate seems to depend upon this conversion of the pectic colloids from a less hydrophilic to a more hydrophilic state. During the soaking there is a loss of material through solution of the pectins. This amounted in one case to 14.37 per cent of the original dry weight. Because of the loss of the most hydrophilic constituents by solution during the experiment, it is impossible to secure the same results with tissues which have been soaked for a number of days and then redried. The substances most responsible for the behavior have been lost during the first treatment.

There may be other causes of irregularity in absorption behavior, but those we have found thus far seem to fall into the four categories which have been described and illustrated by examples.

Discussion

From experiences extending through a long period of years, it seems safe to say that perfectly regular absorption behavior of dry plant material in the presence of water is seldom observed. One has to use unusual care to secure data uniform enough to make mathematical analysis profitable. It requires homogeneous material, material structurally able to withstand the strains of unequal expansion without breaking, and very closely controlled manipulation to secure examples of regularly decreasing rates of water absorption with time.

This paper considers a few of the irregularities of behavior which confront the investigator of water relations during absorption. We include some repetitions and extensions of experiments which gave a great deal of trouble in our earlier work, when laws of absorption were under examination. The results of our experiments, and the attempted explanations, are offered in the hope that plant physiologists will not call the secondary increases in total absorption, or the temporary increases in absorption rates sometimes registered, "double maxima." As OHGA (5) has noted, we were perfectly aware

[3] These tests were made by Mr. RALPH CAIRD and Mr. MEYER HALUSHKA under the direction of Dr. S. V. EATON.

of this secondary increase, but carefully avoided calling it a "double maximum" because we felt that such a term was first of all a misnomer, and secondly that it implied some unknown but mysteriously significant behavior. If we know the causes of physiological behavior we can get along very well in our philosophical discussions of behavior without such terms as "double optima" and "double maxima." Such terms are appropriate to stimulus physiology and the philosophy which has been developed in that field, but are not necessary when causes can be surely established.

OHGA'S results with lotus fruits are in no way different from ours with peas or corn. In these cases the cracking can occur at almost any time after the outer regions become swollen. It depends upon the coherence, the degree of resistance to strain, in the seed substance. Since the breaks occur at irregular times, and not all at once, the curves will show considerable difference in detail.

To the mathematician, moreover, there is no double maximum in these curves. The most rapid intake usually occurs during the first infinitesimal time unit. The point of origin of the rate curve is high up on the axis of ordinates. This point is not properly called a "maximum." The curve drops rapidly with time, and approaches the axis of abscissae. But when the period of breaking arrives, or when some other irregular increase in rate occurs, the curve shows a minimum as the rate rises. At the close of this rise a maximum is established, after which the rate falls to zero with saturation. So far as a mathematical treatment is concerned, there is just one minimum and one maximum in such a curve, and it is improper to call it a "double maximum."

To illustrate the mathematical use of the terms minimum and maximum, figure 13 has been prepared. Curve *A* has a single minimum but no maximum point, while curve *C* has a single maximum but no minimum. Curve *B* has neither maximum nor minimum. Curve *E* is the type that would be necessary to show a double maximum, as at x and x_1. It also shows a double minimum. Curve *D* is the type we are dealing with in these irregularity problems, and it also represents the type of rate-change curve applicable to OHGA'S results with lotus seeds. It has a minimum followed by a maximum, but not a "double maximum."

There are also mathematical objections to OHGA's use of the term "rate" for his determinations. He uses the total intake for each half-hour period to establish a "rate" curve. It is only when the rate of a process is *constant* that the total increase in weight during a definite period of time accurately expresses the rate of the process.

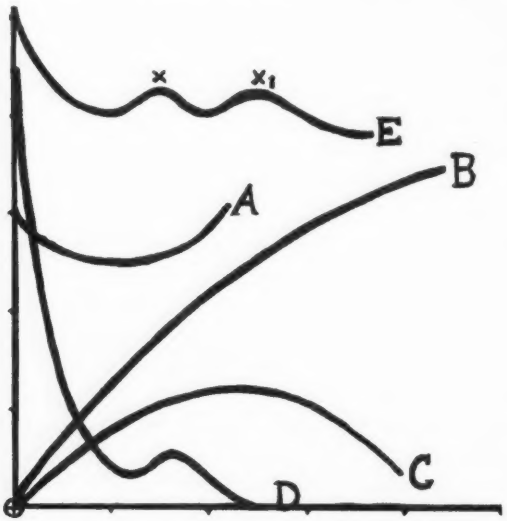


FIG. 13.—Types of curves to illustrate the mathematical meaning of the terms minimum, maximum, "double maximum," etc.: *A*, curve with single minimum; *B*, curve with neither minimum nor maximum; *C*, curve with maximum only; *D*, curve of rate similar to those discussed in this paper (with one minimum and one maximum); *E*, curve showing "double maximum" at x and x_1.

When the rate is not constant, as in absorption, the time periods must be made infinitesimal to measure the rate at a given moment. We made the time periods infinitesimal in the work quoted by OHGA (5). In figure 3 of this paper, in which the relative gain per minute in weight of pea cotyledons is given, the values used are faulty for this same reason. The gain during a fixed period of time not infinitesimal *does not measure the rate*, but approximates it more and more closely as the time periods are shortened. We have used time periods short enough in figure 3 to reveal the type of curve, with its

irregular hump; but it is not an accurate rate curve. If one were to choose other time periods than those chosen by OHGA, one could arrive at different conclusions. HAASIS (**3**, p. 390) called attention to the different types of curves obtained by using different time periods in physiological studies. The way to obviate all difficulties is to plot total intakes with time and then calculate the rate for any infinitesimal period desired, from the tangent to the curve at that moment. Any other method is dangerous when the rate is constantly changing. It was for this reason that we used mathematical formulae and calculated tangents in all of our work where the regularity of behavior permitted or warranted it. We felt it necessary to substitute the refinement of mathematical calculations for the crudities of the graphic method. Here we are using graphic methods because the behavior is too erratic to make mathematical analysis of any value.

Since the hump on the rate curve such as is seen on curve *D*, figure 13, can occur at various times, may be of variable size, and never twice exactly alike in the same kind of materials, we prefer to call the change in rate merely an irregularity. OHGA's lotus seeds present another interesting case of irregularity, most probably caused by internal cracking of the cotyledons during unequal swelling.

There are other possibilities, of course, such as non-homogeneity, or hydrolysis of colloids. OHGA suggests only that it may be connected with the thickness of the material, and in this he is probably correct, as thick materials provide conditions for cracking under stress. We have not had an opportunity to investigate them. At any rate one can say very definitely that there is nothing mysterious about the cases we have examined. The irregular behavior always results from obvious causes, and plant physiologists do not yet need to reckon with "double maxima" in connection with water absorption, whatever may be the situation with reference to other processes (**3**, **4**, **10**).

Summary

1. This paper considers the irregularities in absorption rates of various types of plant materials. Cotyledons of peas, corn grains,

seeds of *Xanthium*, cotton, and rose mallows, and tissues of *Auricularia* have been used in the experiments.

2. The observed irregularities are ascribed to various causes, and fall into four categories. Other causes of irregular behavior may exist, but the majority of them can be placed within the categories named.

3. One of the main causes of secondary increases in absorption rate is the breaking of the interior of the material by unequal swelling. This type of behavior is illustrated by pea cotyledons and corn grains.

4. Occasionally irregular behavior is caused by the development of osmotically active solutions within differentially permeable membranes. *Xanthium* seeds in the presence of acetic acid or alkaline solutions usually exhibit this type of irregularity.

5. Lack of homogeneity has been found to cause irregularities in rate curves. So-called "hard" seeds of certain of the Leguminosae and Malvaceae illustrate this type.

6. Hydrolysis of colloidal substances of pectic nature, as in the case of *Auricularia* tissues, occasionally is the cause of irregularity in absorption rates.

7. Objections are raised to the use of the term "double maximum" in describing such cases of irregularity, first because there is really only one maximum on the rate curve; and second because such a term connotes an unknown or mysterious significance which is unscientific.

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THE SEEDLING ANATOMY OF IPOMOEA BATATAS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 436

HERMAN E. HAYWARD

(WITH PLATES VI, VII AND TWELVE FIGURES)

The sweet potato, *Ipomoea batatas* Lam., is the only representative of the Convolvulaceae of major importance as a food plant. Originally a native of tropical America and the West Indies, its many varieties are now widely cultivated in the south Atlantic and Gulf states, South America, Africa, Mediterranean Europe, India, Japan, the Malay Archipelago, Australia, and New Zealand. Because of its economic importance, anatomical studies have centered upon the mature plant with emphasis upon root development, vegetative propagation, and abnormal anatomy resulting from physiological and pathological conditions.

GROTH (4), KAMERLING (9), MCCORMICK (13), PETERSEN (14), ARTSCHWAGER (1), and others have investigated the root and stem anatomy of this species; BEALS (3) and ISBELL (7) among others have studied phases of vegetative regeneration; and ARTSCHWAGER (1, 2), and HARTER (5) and his co-workers have investigated problems relating to internal breakdown of tissues in storage, formation of wound periderm, stem rot, and other pathological conditions. The present paper supplements the studies previously made with an account of the seed and seedling development of this plant.

FLOWERING HABITS AND SEED PRODUCTION

Under conditions of commercial production in the United States, *Ipomoea batatas* rarely produces flowers, and viable seeds seldom develop. STOUT (17) concluded that sterility had been accentuated through perpetuation of varieties having sterile tendencies for more than 400 years of vegetative propagation; that blooming requires a longer growing season than is necessary for the development of a

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

good crop of roots; and that some type of self-sterility or incompatibility operates to limit seed production even when environmental conditions favor blooming.

THOMPSON (18) at the Virgin Islands carried on extensive breeding experiments over a period of years, and large quantities of seed have been produced there since 1922. He found that untreated seed germinated very irregularly, some within two to four days after planting and others at any time within 100 days. Scarifying the seed improved and hastened germination. Seed production in considerable quantities has also been reported from several other stations, including Queensland, British West Indies, Cuba, the Philippines, and Hawaii.

FRUIT AND SEED[2]

The fruit is a globose capsule which usually consists of a two-celled ovary in each locule of which two ovules are produced. False septa may divide each locule so that the fruit appears to be a four-celled, four-valved capsule with a seed in each locule.

The anatropous ovules are more or less basal and develop in an erect position from axial placentas at the inner angle of each locule. The seed is approximately 3 mm. in its greatest dimension, black, somewhat flattened, with one surface angular and the other roughly convex. The seed coat is tough and corneous, and very impervious to water. The internal structure is similar to that described by LUBBOCK (12) for other species of *Ipomoea* and for *Convolvulus*. The micropyle is deeply invaginated so that a tubular cavity is formed above the hilum (fig. 1*A*). The inner portion of the seed coat is infolded to such an extent that the apical end of the seed is two-chambered (fig. 1*B*). This septum probably determines the configuration of the two deeply bilobed cotyledons.

As the embryo develops, the hypocotyledonary axis comes to occupy the tubular cavity formed by the invagination of the micropyle. The development of the lateral lobes of the cotyledons, after the apex of each has come in contact with the intruded septum, re-

[2] The seeds used were of the Wrenchy and mixed varieties and were obtained from the 1931 crop raised at St. Croix, Virgin Islands. I am indebted to Mr. JOHN B. THOMPSON, of the Virgin Islands Experiment Station, for his cooperation in supplying this material.

sults in the pushing of half of each cotyledon into one of the lateral chambers and the formation of well defined auricles which lie on either side of the hypocotyl. Continued growth of the cotyledonary

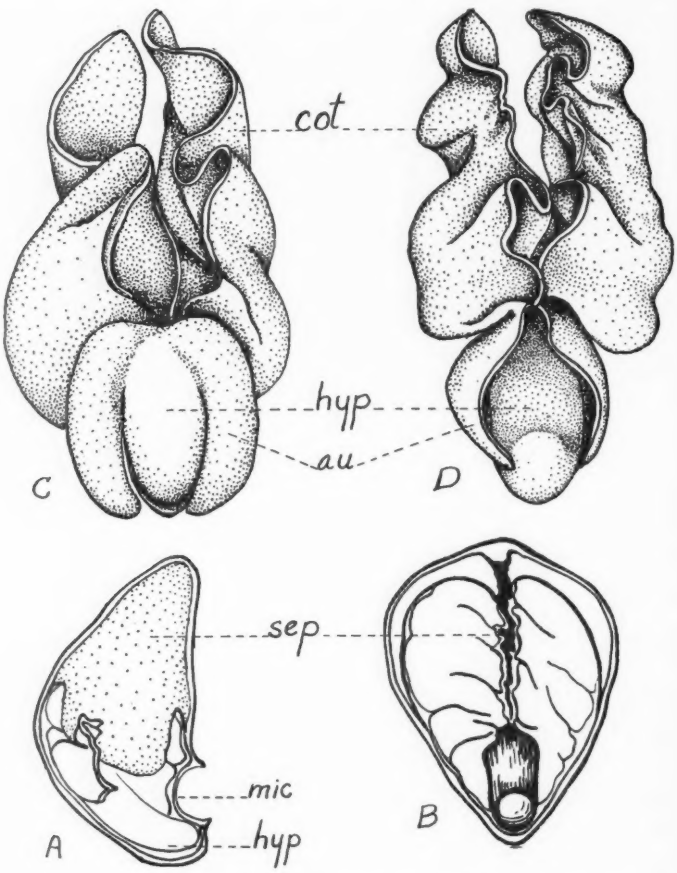


FIG. 1.*—*A*, median view of seed with portion of cotyledons cut away to show septum; *B*, ventral view of same; *C*, dorsal aspect of dissected embryo after 24 hours; *D*, ventral aspect of same: *sep*, septum; *mic*, micropyle; *hyp*, hypocotyl; *cot*, cotyledon; *au*, auricle.

* Figures 1 and 2 were drawn by Mr. SEVILLE FLOWERS.

lobes results in their becoming transversely folded. The dorsal and ventral aspects of the dissected embryo are shown in figure 1 *C* and *D*. The seeds are albuminous, the pulpy endosperm overlying the folded cotyledons. Upon germination this becomes mucilaginous and is absorbed by the cotyledons.

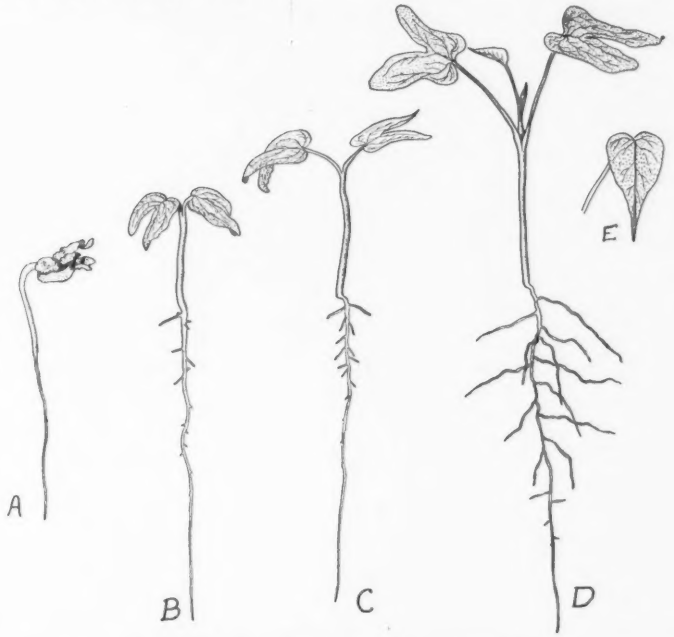


FIG. 2.—Development of seedling: *A*, three days; *B*, five days; *C*, seven days; *D*, three weeks; *E*, first foliage leaf. ×⅔.

GERMINATION OF THE SEEDLING

Under greenhouse conditions scarified seeds germinate rapidly. The primary root emerges from the seed coat by the end of the first or second day. Rapid elongation takes place, so that by the third or fourth day it may reach a length of two or three inches (fig. 2 *A*). There is an early differentiation of laterals, and by the fifth day small branch roots can be seen at the upper root levels (fig. 2 *B*). The hypocotyl elongates, carrying the cotyledons forward with it. As they

emerge from the soil and are liberated from the seed coat, they draw apart and the elongation of the cotyledonary petioles separates them from each other (fig. 2*C*). The cotyledons are deeply bilobed, the lobes being obtuse or subacute, and the rounded auricles are rather prominent.

Following the expansion of the cotyledons, the epicotyl begins to elongate and the first foliage leaves expand (fig. 2*D*). In seedlings 21 days old there may be three or four expanded foliage leaves. These are simple, cordate at the base, and acute or pointed at the tip (fig. 2*E*).

PRIMARY ROOT

The primary root is tetrarch, with four groups of small phloem cells alternating with the xylem points and separated from them by intervening fundamental parenchyma (fig. 3). Each point consists of five or six protoxylem elements, the outermost and smallest element abutting on a single layered pericycle and the larger innermost ones on a zone of parenchyma cells. The central cell of this zone is a much enlarged metaxylem element. A somewhat well defined endodermis surrounds the pericycle and limits the cortex centripetally. The cortical cells are large, with prominent intercellular spaces and are limited externally by the epidermis.

The development of the root axis resembles that described by JANCZEWSKI (**8**) for *Linum usitatissimum*. The meristem is differentiated into three regions. The plerome and periblem which give rise to stele and cortex respectively are clearly defined, and a layer of cells, the calyptrogen, overlying the periblem produces the root cap and epidermis (fig. 4).

The root cap consists of several layers of cells which overlie one another in vertical series, thus indicating their direct origin from the calyptrogen layer. The cells of the root cap divide more frequently at the tip than at the base, so that the cap is thicker at its apex and diminishes toward its base. This differential activity results in the formation of several vertical rows of cells in the central portion of the cap (fig. 4). Growth and subsequent divisions of the cells of the cap frequently result in irregularities in the lateral regions of the same.

The epidermis does not arise from an independent histogen, but is derived from the calyptrogen layer. Those cells of the calyptrogen which have become laterally oriented, due to the growth of the underlying periblem and plerome, divide less frequently than those

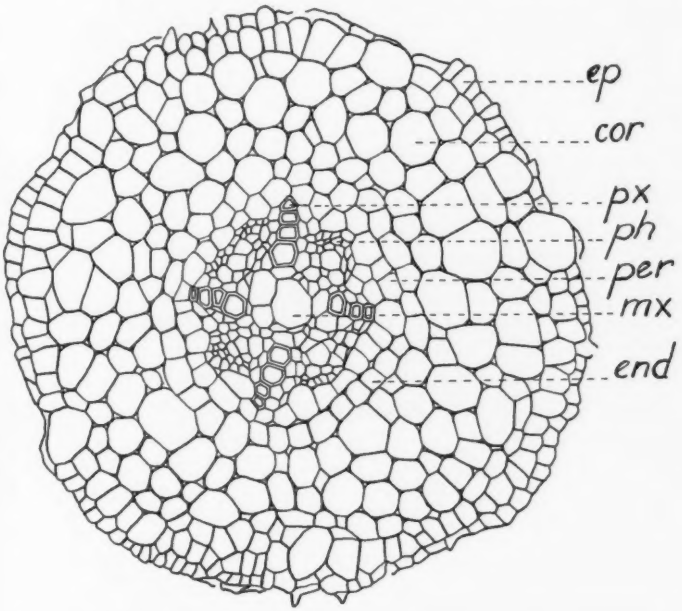


FIG. 3.*—Primary root, transection; metaxylem not completely differentiated: *ep*, epidermis; *cor*, cortex; *end*, endodermis; *per*, pericycle; *ph*, phloem; *px*, protoxylem; *mx*, metaxylem.

* Figures 3, 4, and 11–18 were drawn by Mr. T. K. YEN.

at the tip. The final periclinal division of these laterally placed cells results in two cells, the inner one of which becomes an epidermal cell and the outer one of which contributes to the root cap (fig. 4). In this manner successive additions are made to the epidermis as growth in length of the root proceeds, and, because of it, the outer surface of the epidermal layer appears in median longisection as a series of steps, each of which rests upon a layer of the root cap.

The cortex is derived from the periblem, which consists of two layers of cells overlying the plerome. The outermost layer divides only anticlinally and forms the outer layer of the cortex, while the

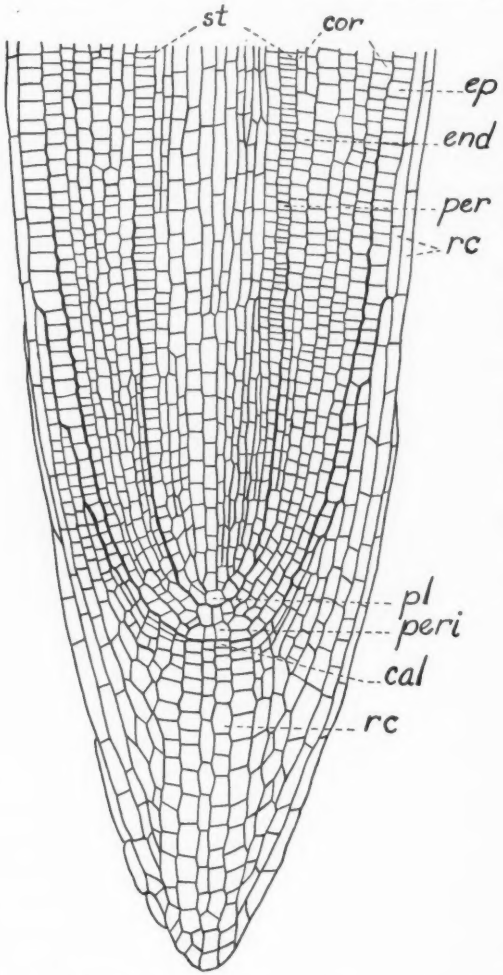


FIG. 4.—Median longisection of primary root: *ep*, epidermis; *cor*, cortex; *st*, stele; *end*, endodermis; *per*, pericycle; *pl*, plerome; *peri*, periblem, *cal*, calyptrogen, *rc*, root cap.

inner layer divides in all planes to form the remainder of the cortex which at maturity is six to nine cells in thickness (fig. 4).

The stele is differentiated from a small group of plerome cells lying above the periblem. The lateral members of this group produce the pericycle which can be distinguished from the other regions of the stele early in ontogeny, since the cells composing it are radially rather than vertically elongated. In the development of the vascular portion of the stele, the primary phloem is first differentiated and four groups of vertically elongated phloem elements are formed by longitudinal divisions of plerome cells lying immediately within the pericycle. Coincident with this development, four primary latex vessels abutting on the pericycle are formed by the enlargement of a single vertical row of phloem cells in each group (fig. 5). The cells of these vessels retain their end walls and protoplasts. The primary phloem consists of very slender elongated sieve tubes and companion cells. The sieve plates are commonly transverse but may occasionally be obliquely oriented.

The primary xylem differentiates centripetally. The first protoxylem cells to mature are thin-walled with annular or annular-spiral thickenings. While the protoxylem cells are differentiating, a vertical row of fundamental parenchyma cells in the center of the stele becomes much enlarged to form a single metaxylem vessel. This vessel is usually separated from the adjacent metaxylem and the protoxylem points by a zone of parenchyma cells. In some instances two central metaxylem vessels may be formed, one of which is usually larger than the other. The outermost metaxylem elements have loosely reticulate secondary wall thickenings while the centrally located metaxylem element is closely reticulate. At the upper root levels the center of the stele is parenchymatous.

Lateral roots are formed very early in the ontogeny of the primary root. Even before the protoxylem is completely differentiated, the development of laterals may be initiated in the cells of the pericycle lying directly outside the xylem points (fig. 6). Tangential and radial divisions of the pericyclic cells result in the formation of a conical group of meristematic cells or growing point, and the further development of the lateral root is similar to that described for the primary root itself. The laterals are usually tetrarch but may be

triarch. Pentarch, hexarch, and other types found in roots of *Ipomoea batatas* which have been grown vegetatively were not observed in the seedling material studied but it is not unlikely that such types may occur.

Transition in the hypocotyl

The lower portion of the hypocotyl is rootlike in general plan. The primary xylem and phloem are alternate and radial but the central portion of the stele is parenchymatous. At successively higher levels near the middle of the hypocotyl there is a gradual reorientation of the primary vascular tissue. The four phloem regions are divided into four wide arcs consisting of scattered groups of phloem cells which lie immediately inside the pericycle and between the primary xylem points. The metaxylem elements differentiate in an intermediate or tangential position with respect to the protoxylem, instead of radially as in the root, so that each of the four xylem poles consists of the protoxylem point with two tangential arms of intermediate metaxylem (fig. 7).

In the upper hypocotyl, the four arcs of scattered phloem groups are tangentially extended so that the lateral members of each arc superpose the metaxylem. The outer phloem thus forms a discontinuous ring of elements some of which are collateral with the metaxylem. The first groups of internal phloem are differentiated in the pith and lie between the two laterally divergent arms of the metaxylem of each xylem pole (fig. 8). The internal phloem is not continuous with the primary phloem of the root. It arises later in ontogeny than the outer phloem of the hypocotyl, which is continuous with that of the root, and consists of strands which have differentiated downwardly from the cotyledonary plate as continuations of the internal phloem of the bicollateral bundles of the cotyledonary traces.

There is usually no connection between the outer and inner phloem, and the strands of the latter end blindly in the pith of the hypocotyl. The xylem point shown in figure 11 is one located below the level at which the metaxylem groups become separated. In this case no interconnection between the outer and inner phloem occurred above or below the level figured. In a few instances the inner

and the outer phloem appeared to be transversely connected by differentiation of the parenchyma cells lying in the gap between the divergent and partially separated arms of intermediate xylem.

In the upper hypocotyl the cotyledonary strands may be distinguished from the intercotyledonary on the basis of the degree of separation of the two metaxylem groups of each strand. Those of the intercotyledonary strands become more widely separated from each other than those of the cotyledonary strands, and the orientation of the cotyledons may thus be determined below the level where the hypocotyl becomes oval in outline.

Slightly below the cotyledonary node, the metaxylem groups of each intercotyledonary strand are more widely separated, each group inclining toward one of the cotyledonary strands. At this level the internal phloem is well developed, so that there are two bicollateral polar bundles of the double bundle type and four smaller laterally placed bicollateral bundles derived from the intercotyledonary strands (fig. 9).

At the cotyledonary node, the two lateral bundles of each cotyledonary trace abut the right and left flanks of the median double bundle of the trace so that it appears to be a single large crescentic unit. Slightly above the level of divergence of the cotyledonary traces from the vascular ring, the lateral bundles of each trace separate from the median bundle which is itself divided to form two bicollateral bundles (fig. 10). The xylem of these bundles is not endarch in its development but consists of tangentially oriented metaxylem lying between the outer and inner phloem. Between the two median bundles is a single protoxylem strand.

Cotyledons

The slender cotyledonary petiole equals or slightly exceeds the lamina in length, and its adaxial surface is grooved so that it is crescentic in cross-section. At its base there are four bundles. The two large median bundles are bicollateral, the outer phloem consisting of several scattered groups of cells and the inner of two or three such groups. In some instances the xylem may be surrounded by a discontinuous ring of phloem strands so that the bundle is essentially amphicribral. The two smaller lateral bundles are collateral

or half amphicribral (fig. 12). The parenchyma cells surrounding the bundles are thin-walled and loosely arranged, with large intercellular spaces. Toward the abaxial surface, the outer cell layers contain chloroplasts and are compact and collenchymatous in character, with thickened angles. The subepidermal cells of the concave adaxial

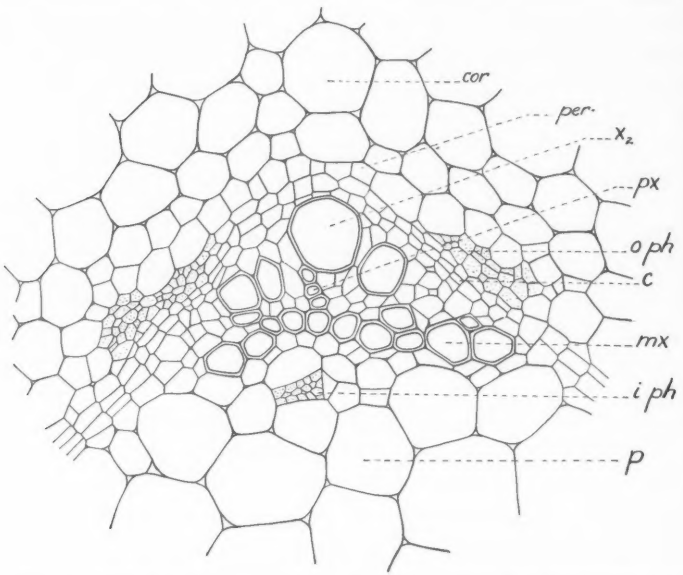


FIG. 11.—Details of a protoxylem point in lower hypocotyl of 21-day seedling: *cor*, cortex; *per*, pericycle; *px*, protoxylem; *mx*, metaxylem; x_2, secondary xylem; *oph*, outer phloem; *iph*, inner phloem; *c*, cambium; *p*, pith.

surface are also chlorophyllose but not so compactly arranged, since the more numerous stomata open into large intercellular spaces.

The arrangement of the petiolar bundles remains unchanged up to a point slightly below the lamina, where the two median bundles anastomose to form one broad bundle (fig. 13*A*). At the base of the lamina the two small bundles (a, a') curve abruptly outward and supply the auricles. The median bundle cuts off two lateral branches (b, b') which immediately give rise to two small laterals (c, c') that supply the basal portion of the lamina. The median bundle finally

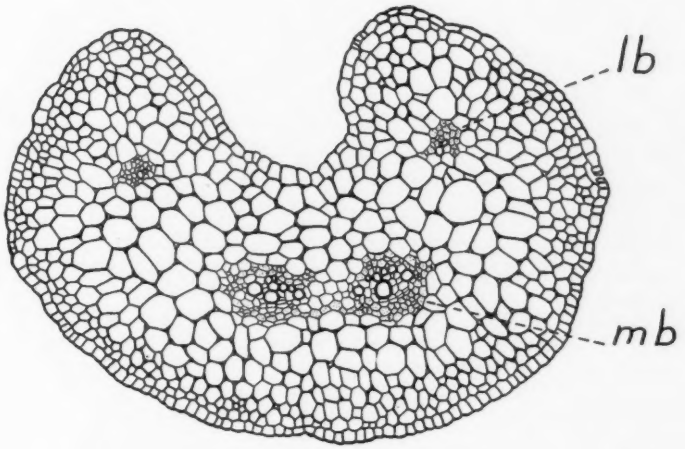


FIG. 12.—Cotyledonary petiole, transection: *lb*, lateral bundle; *mb*, median bundle. Adaxial surface of petiole is oriented toward the top.

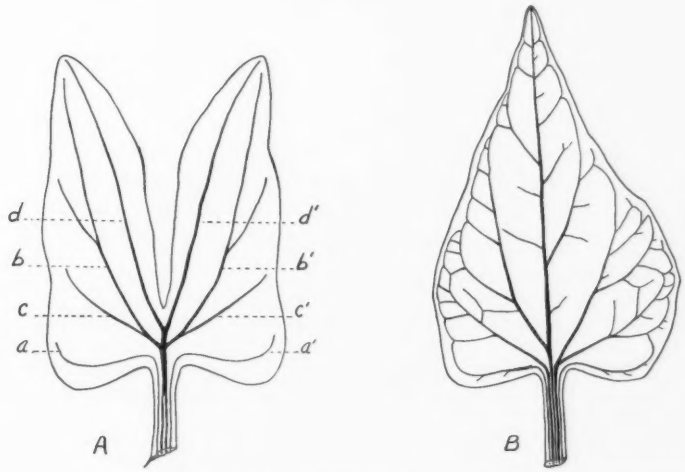


FIG. 13.—*A*, diagram of mature cotyledon showing venation of lamina and petiole; *B*, diagram of foliage leaf showing venation.

is forked just below the notch of the blade, one branch extending into each lobe (d, d'). These with the branches b and b' supply the lobes of the cotyledon.

The median bundle is still bicollateral at the base of the lamina, but the inner (adaxial) phloem ends blindly in the mesophyll before the strand forks. The other veins are collateral throughout their extent in the blade, and there is a progressive reduction in the amount of phloem tissue in succeeding branches until the ultimate veinlets consist of a single spiral tracheid.

The primary xylem, which is tangentially oriented in the petiole, is differentiated in a manner equivalent to the endarch condition in stems with the protoxylem elements toward the upper surface of the cotyledon.

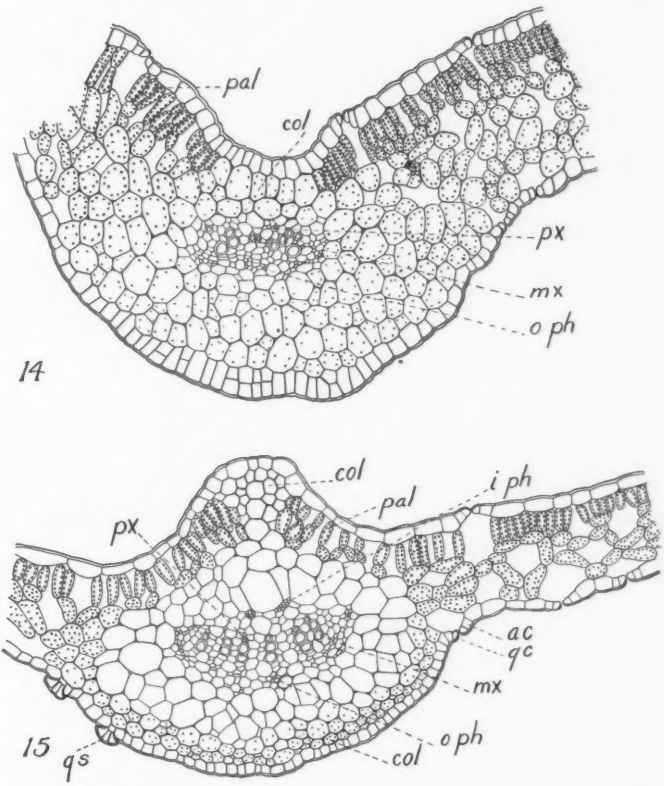
The cells of the upper epidermis of the lamina are larger than those of the lower and are somewhat convex on their outer surfaces and irregular in size, so that the surface is roughened. Stomata occur in both epidermal layers but are more abundant in the lower. In the mesophyll the palisade parenchyma is loosely organized, and usually consists of two rows of cells with occasional groups of longer cells interspersed whose length equals that of the double palisade. The spongy tissue is four or five cells in thickness except at points where the principal veins traverse it. The spongy cells are isodiametric and so arranged that large lacunae and substomatal cavities are formed. In the region of the larger veins the mesophyll is more compact, the palisade tissue and spongy cells being replaced by collenchyma cells above and below the vein (fig. 14). Glandular scales or hairs occur on the lower epidermis. These consist of a basal cell subtending several elongated cells that are arranged in a disk to form the flattened or convex surface of the scale.

Development of the foliage leaves

The growing point of the epicotyl cuts off leaves which are spirally arranged with a two-fifths phyllotaxy. At the time of differentiation of the first foliage leaves, the epicotyledonary axis is surrounded by the bases of the cotyledons which form a partial sheath or cotyledonary collar.

In the ontogeny of the leaf, the leaf primordium arises from the

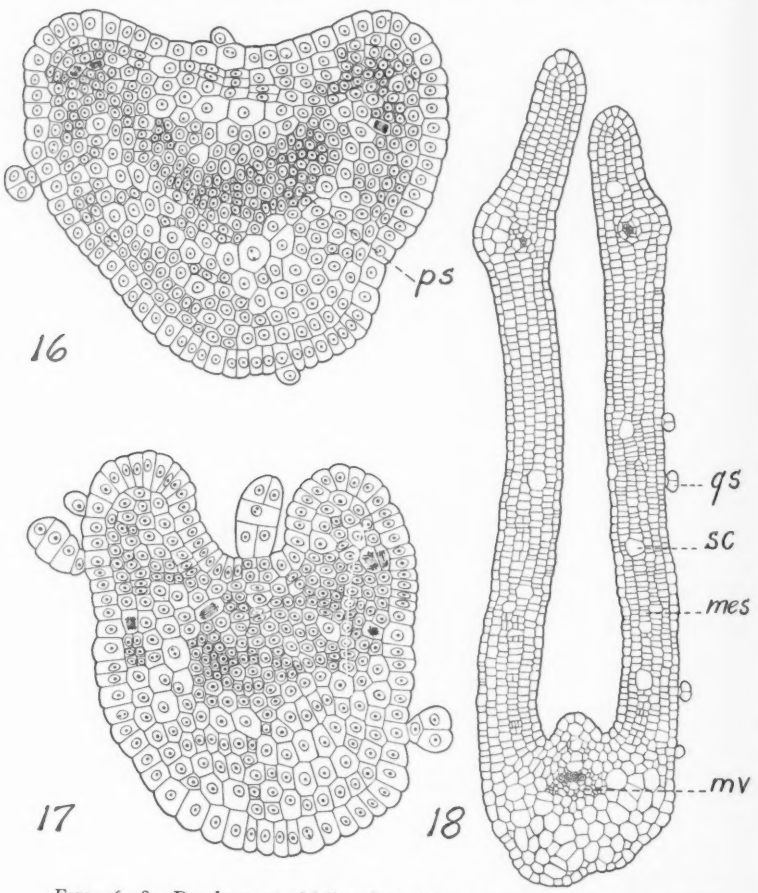
surface tissues of the growing point as a conical mass of cells. The primordium elongates and broadens by general meristematic ac-



FIGS. 14, 15.—Fig. 14, section of lamina of cotyledon through midvein: *pal*, palisade tissue; *col*, collenchyma; *px*, protoxylem; *mx*, metaxylem; *oph*, outer (abaxial) phloem. Fig. 15, Transection of foliage leaf through midrib near base: *pal*, palisade tissue; *col*, collenchyma; *ac*, accessory cell; *gc*, guard cell; *px*, protoxylem; *mx*, metaxylem; *iph*, inner (adaxial) phloem; *oph*, outer (abaxial) phloem; *gs*, glandular scale.

tivity until it becomes somewhat crescent-shaped or triangular in cross-section at the base (fig. 16), tapering to a rounded or slightly subtriangular apex. There is early differentiation of the procambial

strands in the basal portion of the primordium which later form the vascular tissue of the petiole of the leaf. Growth in length of the



FIGS. 16–18.—Development of foliage leaf: Fig. 16, transection of young leaf primordium near base showing procambial strands (*ps*) and region of meristematic activity. Fig. 17, transection of same primordium at slightly higher level showing development of lamina as result of accelerated meristematic activity on lateral portions of adaxial surface. Fig. 18, transection of young leaf showing orientation of lamina with respect to midrib: *gs*, glandular scale; *sc*, secretory cell; *mes*, mesophyll; *mv*, midvein. Figures 16 and 17 magnified to twice size of figure 18.

primordium is accomplished by cell division, but there is an acceleration of meristematic activity on the lateral portions of the adaxial surface (fig. 17). This activity results in the formation of the lamina which develops with the upper surfaces of the two halves of the blade opposing each other. They are thus oriented at right angles to the midrib with their edges directed toward the axis of the epicotyl (fig. 18). Following differentiation of the lamina, the petiole elongates by continued cell division and enlargement.

The epidermal tissue of the developing leaf produces glandular scales which are more numerous on the exposed lower epidermis than on the upper. The mesophyll is at first very compact, with little differentiation into spongy and palisade regions; but later in ontogeny the spongy tissue becomes loosely organized, with large substomatal cavities and intercellular spaces. The palisade tissue remains relatively compact except where it is interrupted by substomatal cavities, which are less numerous than in the spongy tissue.

The palisade consists of one to three layers of palisade parenchyma. There are occasional series of transversely divided parenchyma cells intervening between the typical palisade parenchyma. In the young leaf large secretory cells are evident, lying between the palisade and spongy tissue; but these become much less conspicuous as maturation proceeds and the adjacent cells enlarge. These latex cells may become non-functional as such in the mature leaf and in that case persist as air chambers.

At maturity the cells of the mesophyll are much less compact, so that there are numerous large intercellular spaces in the spongy region and between the palisade cells. The latex cells are not easily detected, and resemble large air cavities which in most instances are not directly connected with adjacent air chambers owing to the retention of the original cell wall.

The epidermal cells are irregular in size and develop a definite cuticle. The stomata are more numerous in the lower than in the upper epidermis, and the guard cells are commonly subtended by accessory cells. In the region of the midrib and the principal lateral veins, the palisade gives way to mechanical tissue above the vein which forms a projecting ridge on the upper surface. Below the vein a zone of collenchyma cells develops which further reinforces the

leaf at these points. The midrib is bicollateral in character at the base of the leaf; but there is a gradual reduction in the amount of internal (adaxial) phloem, until finally the collateral condition is reached (fig. 15). The lateral veins are collateral in character. As they branch and rebranch to form the small veins and veinlets, there is a gradual reduction in the amount of outer (abaxial) phloem. Finally the xylem is reduced until the ultimate veinlets terminate as single spiral tracheids which end blindly in the mesophyll.

At the base of the leaf there are five principal veins which proceed for some distance down the petiole without anastomosing (fig. 13*B*). The three median ones are bicollateral, while the two laterally placed ones are half-amphicribral or collateral in character. At the base of the petiole the two laterally placed bundles anastomose with the right and left median bundles, so that the leaf trace consists of two large lateral bundles and a smaller median one. The downwardly diverging leaf traces of the first, second, and third foliage leaves constitute the primary vascular tissue of the first internode, so that at the time of maturity of the primary vascular tissue, this portion of the epicotyl consists of eight or nine bicollateral endarch bundles, which are the downward extensions of the leaf traces of the first foliage leaves. Near the cotyledonary plate these converge to form two lateral zones of vascular tissue which anastomose with the lateral bundles of the cotyledonary traces in the intercotyledonary plane.

Discussion

In the Convolvulaceae the relationship of the internal phloem to the external phloem has received considerable attention and has been variously interpreted by investigators working with members of this group.

SCOTT (**15**) in his investigation of *Ipomoea versicolor* Meissn. states that "the internal phloem extends downwards into the hypocotyl and passes out between the converging protoxylem groups of each cotyledonary pair of bundles, thus joining the external phloem of the root." In his conclusion he further states, "As regards the course of the internal phloem in the transition from stem to root, *Ipomoea versicolor* may be taken as typical of plants with bicollateral bundles generally."

LEE (**11**) investigated the seedling anatomy of *Convolvulus tricolor* L. var. *major* and also *C. tricolor* L. In regard to the former he states: "The phloem is extremely small in amount and at no point can internal phloem be recognized with certainty—an observation which emphasizes the late appearance of this tissue noticed by other observers." In the case of the latter, he notes frequent anastomoses of strands of phloem connecting the bundles in the petiole of the cotyledon, and also calls attention to the abundance of internal phloem in the petiole and hypocotyl. In this instance he agrees with SCOTT, stating that "in the upper part of the hypocotyl, during the rearrangements in the cotyledonary strand so that the protoxylem becomes external, the internal phloem masses of that bundle fuse, and the fused mass gradually passes out between the metaxylem groups (before the latter meet to form the root bundles) and joins on to the external phloem. The same events take place at a lower level in connexion with the inter-cotyledonary bundles, so that finally an ordinary tetrarch root is produced."

LAMOUNETTE (**10**) investigated several of the Convolvulaceae, including *Ipomoea leucantha*, and concluded that the internal phloem when present in the hypocotyl is differentiated in the medullary parenchyma and is not continuous with the external phloem of the root. In fact, he rejects the term bicollateral, supporting HÉRAIL (**6**) in this regard.

In the present investigation anastomoses between internal and external phloem were observed at the base of the cotyledonary petiole, but no evidence was found to indicate that the internal phloem of the hypocotyl regularly anastomosed with the external phloem by passing between the protoxylem points.

Strands of internal phloem were traced downward in the hypocotyl, to a point below the level at which the divergent metaxylem arms become confluent with the protoxylem to form the root bundle, without finding evidence of interconnection between outer and inner phloem; and it appears that in this species the internal phloem ends blindly in the medulla of the lower portion of the hypocotyl. All workers in this group have agreed that the internal phloem develops later in ontogeny than the external; and SCOTT and BREBNER (**16**) suggest that the lack of agreement regarding the internal phloem

may in some cases be due to the fact that very young seedlings were used, in which the internal phloem was not fully differentiated. In the present study seedlings from two days to three weeks' old were used. In seedlings 21 days old the axis is developed to a point where the primary xylem is completely differentiated and there is an appreciable amount of secondary thickening due to cambial activity.

The internal phloem in *Ipomoea batatas* is therefore regarded as supplementary to the outer phloem but not directly or regularly connected with it by anastomoses, although this may occasionally occur. The late appearance of the internal phloem at a time when the seedling is beginning to function photosynthetically suggests a correlation between the function of the phloem and its anatomical development. This problem is now under investigation.

Summary

1. The structure of the seed and the development of the seedling of *Ipomoea batatas* are described.

2. The primary root is tetrarch, and in the growing point three histogens may be observed. The plerome and periblem give rise to stele and cortex respectively, while the calyptrogen produces the root cap and by a final periclinal division forms the epidermis.

3. In the development of the root, primary phloem is first differentiated and the enlargement of a single row of cells produces four primary latex vessels.

4. Differentiation of lateral roots occurs early in ontogeny before the complete maturation of the protoxylem elements.

5. In transition the primary xylem strands in the cotyledonary plane constitute the xylem of the midvein of the cotyledonary trace; while the intercotyledonary strands, by separation, form the xylem of the lateral bundles of the cotyledonary trace.

6. The internal phloem arises later in ontogeny than the external and is not regularly connected with the external phloem by anastomoses. Some interconnection between the outer and inner phloem was observed in the petiolar region of the cotyledons.

7. Infrequent cross-connection of inner and outer phloem by differentiation of the intervening fundamental parenchyma cells was observed in the hypocotyl.

8. A possible correlation between the degree of development of internal phloem and increasing photosynthetic activity is suggested.

9. The development of the epicotyl and foliage leaves is described.

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EXPLANATION OF PLATES VI, VII

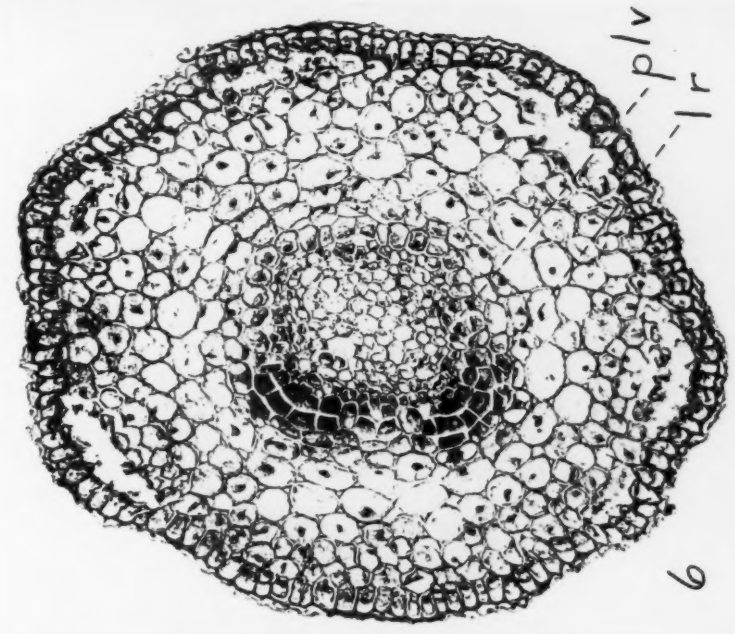
PLATE VI

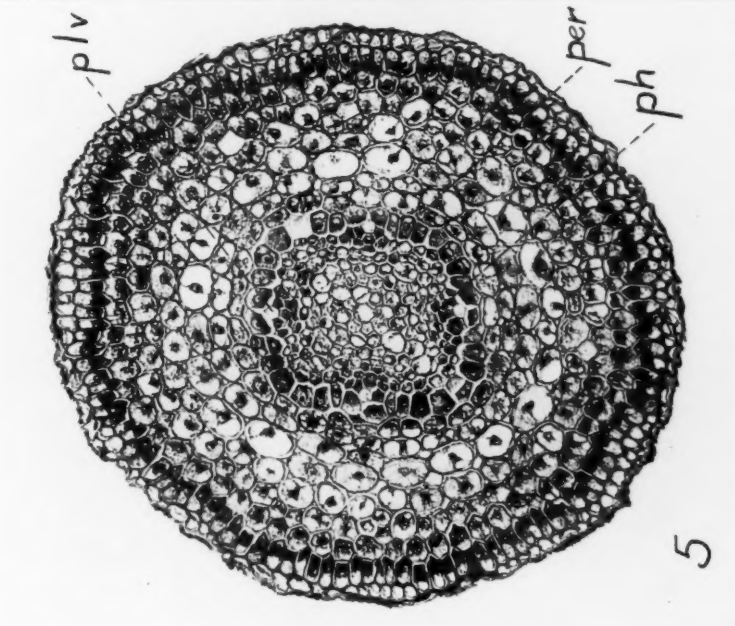
FIG. 5.—Microphotograph, transection of young primary root in region of differentiation showing primary latex vessel (*plv*), pericycle (*per*), and primary phloem (*ph*).

FIG. 6.—Microphotograph of young primary root showing origin of laterals (*lr*).

PLATE VII

FIGS. 7–10.—Transition in hypocotyl: Fig. 7, transection of lower portion of hypocotyl showing tangentially oriented metaxylem arms. Fig. 8, transection through upper portion of hypocotyl showing further divergence of metaxylem and appearance of internal phloem. Fig. 9, transection slightly below cotyledonary node showing median or polar bundles and lateral bundles. Fig. 10, transection slightly above cotyledonary node showing cotyledonary traces consisting of two median bundles and two laterals; cotyledonary collar surrounds the epicotyl. Microphotographs were made from 21-day seedlings: *px*, protoxylem; *mx*, metaxylem; *oph*, outer phloem; *iph*, inner phloem; *lb*, lateral bundles; *mb*, median bundle; *vr*, vascular ring.





HAYWARD on IPOMOEA

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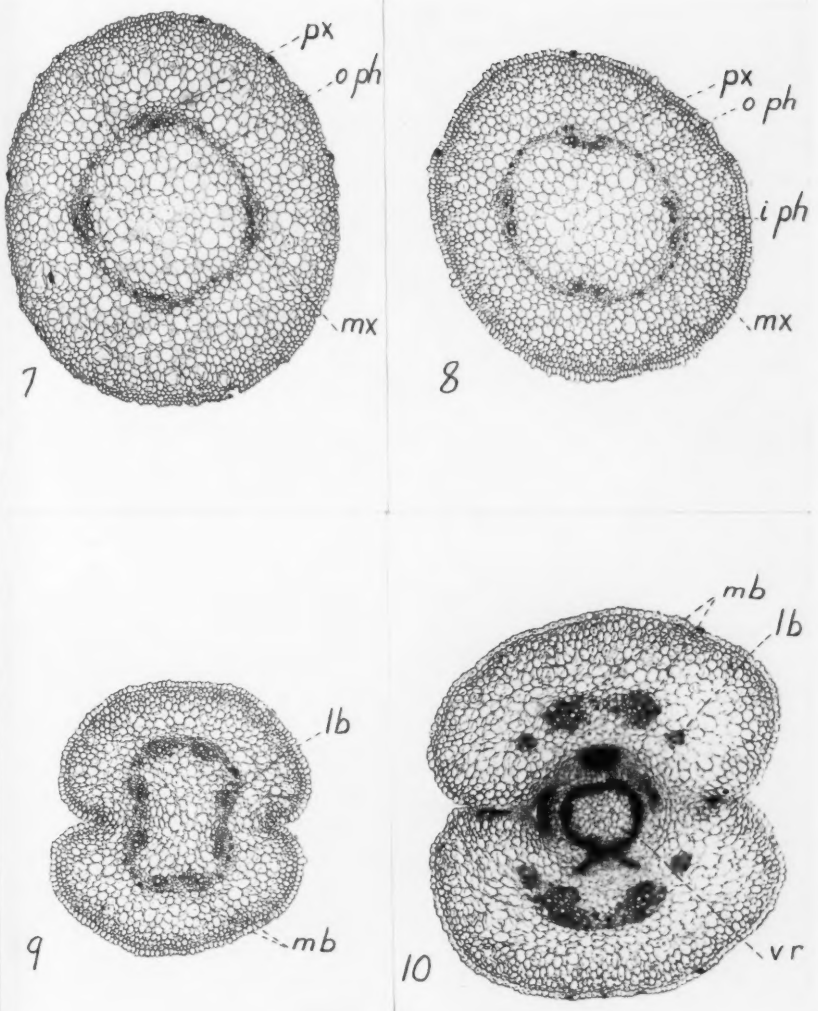

HAYWARD on IPOMOEA

SYSTEMATIC POSITION OF THE SLIME MOLDS AND ITS BEARING ON THE CLASSIFICATION OF THE FUNGI

G. W. MARTIN

Because of their often conspicuous fructifications, the Myxomycetes have long been familiar. The older mycologists quite naturally accepted them as fungi, and while FRIES (**32**, p. 67) recognized the distinction between their plasmodial phase and the mycelium of other forms which he included in the Gasteromycetes, he was content to segregate them as a suborder of that group. It was only as a result of DE BARY'S extensive investigations of the life history of certain of the species that this view was seriously questioned. In his papers of 1858 and 1859 (**3**, **4**), DE BARY emphasized the animal affinities of the slime molds and proposed for them the new name Mycetozoa (fungous animals), which has ever since been widely although far from universally accepted. In the subtitles to these papers he referred to them as "the lowest animals"; in 1864 (**5**) he altered this to "the lowest organisms," thus receding somewhat from his previous extreme position; but as late as 1884 he reiterated his opinion that the slime molds are "outside the limits of the vegetable kingdom" (**7**). Since DE BARY'S time the group has been included almost universally in general works on both botany and zoology.

Zoologists include them in the amoeboid Protozoa. CALKINS (**15**), whose treatment may be taken as representative, places them in the order Euplasmodida, where, with the orders Acrasida and Phytomyxida, they are regarded as constituting the subclass Mycetozoa of the class Rhizopoda, in the subphylum Sarcodina of the Protozoa. In his arrangement within the order he follows the older botanical system, proposed by DE BARY and adopted by SCHRÖTER (**50**). The tendency among mycologists at the present time, however, is to regard the Phytomyxida of CALKINS (the Phytomyxinae of SCHRÖTER) as constituting the family Plasmodiophoraceae of the lower Chytridiales, while the Acrasida are regarded as wholly distinct from either group.

In botanical texts the slime molds are usually included among the Thallophyta, either grouped with the bacteria and fungi as fungi in a wider and more physiological than phyletic sense of the term, or treated as an independent group coordinate with the bacteria and the fungi. It is the latter view that prevails in more advanced treatments. This was extensively elaborated by ZOPF (**56**), who regarded the group, enlarged to include a number of protozoa-like forms, as related equally to plants and animals. This treatment, with the exclusion of most of the animal-like forms, was adopted by SCHRÖTER (**50,** p. 36), who states that the Myxomycetes are unrelated to any other plant group, later partly contradicting himself by admitting that common ancestors of the Myxomycetes and the Chytridiales may possibly be sought among the flagellates. MASSEE (**47**) adopted a similar view. MACBRIDE (**46**) likewise believes them neither plants nor animals, but independent. SCHINZ (**49**) regards them as related to the rhizopods, basing his opinion partly on the earlier work of JAHN. JAHN himself develops this idea more fully in the second edition of ENGLER and PRANTL (**42**), making the Hydromyxales, including the families Plakopodaceae and Vampyrellaceae, the first of the nine orders into which he divides the Myxomycetes. Thus botanists and zoologists agree that the Myxomycetes show evidence of relationship with forms commonly included among the Protozoa. It is generally recognized, of course, that neither the Thallophyta nor the Protozoa are natural phyla, but rather heterogeneous assemblages of plants and plant-like organisms on the one hand, and animals and animal-like forms on the other, held together merely by what is assumed to be the relative simplicity of their members and not by any underlying unity of organization. Naturally there is much overlapping, and if the attempt is to be made to divide all organisms into plants and animals, many forms must be included in both groups. HAECKEL'S proposal that all organisms of doubtful position be united into a single group, Protista, has the merit of convenience for certain purposes, but has failed of general acceptance both in its original form and as modified by various students, since it is felt that combining two already heterogeneous groups into an even more heterogeneous assemblage increases rather than decreases the difficulty of providing an adequate classi-

fication. On the other hand, there has been a tendency to discard the term Thallophyta entirely. This has been done in the systems proposed by ENGLER (**28**), BESSEY (**8**), and CAMPBELL (**16**) among others.

The chief difficulty in the way of regarding the slime molds as fungi is the widely prevalent and often expressed view that the fungi are merely algae, belonging to several algal groups, which have acquired a heterotrophic method of nutrition and have, as a result, lost their chlorophyll. This opinion is traced by ATKINSON (**1**) to KÜTZING and ALEXANDER BRAUN, and was emphasized by PRINGSHEIM, COHN, and SACHS. Its adoption by STRASBURGER and its statement in the earlier editions of his famous text has done much to popularize it, while in the United States the writings of C. E. BESSEY (**8, 9**), E. A. BESSEY (**10**), and CLEMENTS (**21**) have further intrenched it. Very recently CLEMENTS and SHEAR (**22**) have adopted it in its most extreme form, as is evidenced by the statement, "the fungus is a physiological adjustment to the environment and . . . . in consequence fungi are to be found in every major division of the plant kingdom. . . . ."

Since the time of DE BARY, this polyphyletic theory of the origin of the fungi has been opposed by a number of mycologists and at the present time many of them favor a monophyletic system. This was the view favored by DE BARY (**6**). BREFELD (**12**) considered the Ascomycetes and Basidiomycetes derived from the Zygomycetes, while he regarded the Oomycetes as a group of independent origin not having given rise to any higher forms. In his earlier treatment (**11**) he derived the slime molds from the Chytridiales. Many years later (**13**) he declared them to be without related forms among the other fungi.

A second suggestion, that the fungi are derived from colorless amoeboid or flagellated organisms, seems to have first been advanced by CORNU (**25**) and more definitely by GOBI (**37**). It was followed by SOROKIN and has been fully developed by DANGEARD (**26**). The views of these and other students up to 1912 are critically discussed by VUILLEMIN (**54**). ATKINSON (**1**), who was the leading advocate of the monophyletic theory in the United States, believed that either an algal group or colorless flagellates could be postulated as the an-

cestral forms from which the fungi have been derived. CAVERS (**17**), in an extensive and critical review of the literature on the subject, suggested that the Proteomyxa, a group with supposed rhizopod affinities, gave rise to the Myxomycetes, Chytridiales, Plasmodiophorales, and Acrasiales, the higher Phycomycetes having arisen from the chytrids. More recently GÄUMANN (**33**) derives the bulk of the fungi from the green algae, but expressly excepts the lower Chytridiales (the Archimycetes in his restricted sense) from such derivation, believing that they, with the Myxomycetes, arose independently from colorless flagellates. FITZPATRICK (**31**) agrees with CAVERS in rejecting such sharp separation of the lower Chytridiales from the other fungi, and merges the Archimycetes of GÄUMANN in the Chytridiales.

The four families concerned, the Plasmodiophoraceae, Woroninaceae, Olpidiaceae, and Synchytriaceae, are alike in that the thallus, which is parasitic, or sometimes grows in moribund host cells, is (at least during its early stages) naked and somewhat amoeboid. All species are holocarpic and without rhizoids or other trace of a mycelium. The naked character of the thallus is particularly apparent in the first two families, where it persists nearly or quite until spore formation. In the Olpidiaceae and the Synchytriaceae the membrane appears earlier and is finally well developed. FITZPATRICK questions the naked character of the young thallus of *Pseudolpidium* and *Olpidiopsis* as described by CORNU (**25**), although BARRETT'S study of the latter genus (**2**) clearly confirms it, as does direct observation of the living organism. Nevertheless the wide separation proposed by GÄUMANN between these forms and the higher Chytridiales does not seem justified. It would seem that affinities are best expressed neither by this extreme separation nor by completely bridging the gap, but by reverting to an earlier classification into the two orders Myxochytridiales and Mycochytridiales. The first, suggested by GOBI and adopted with modification by FISCHER (**30**), would include all holocarpic forms without rhizoids in which a membrane is lacking or appears relatively late in the development of the thallus, while the second would include the rhizoidal and mainly eucarpic forms surrounded by a distinct membrane from the first.

The preceding summary of the arguments for the monophyletic origin of the fungi is necessary in order to justify the view here presented concerning the position of the Myxomycetes. This is, in brief, that the slime molds may be regarded as constituting an independent and highly specialized class derived from primitive saprobic or holozoic organisms which have also given rise to the lowest Phycomycetes, the Myxochytridiales, from which the remaining fungi have been derived. The fungi would then include four classes of approximately coordinate rank: Myxomycetes, Phycomycetes, Ascomycetes, and Basidiomycetes; or three, if the Ascomycetes and Basidiomycetes be united as suggested by BREFELD. These classes together constitute a phylum, to be included among plants as a matter of convenience, but in reality neither plants nor animals, but an independent group of organisms, one of several such.[1] The Phycomycetes are regarded as composed of three subclasses: the Archimycetes, the Oomycetes, and the Zygomycetes. The Archimycetes include three orders: the Myxochytridiales, the Mycochytridiales, and the Ancylistales. The Myxochytridiales are the lower Chytridiales of FITZPATRICK'S treatment, the Archimycetes of GÄUMANN in his restricted application of the term. They constitute the critical group, since it is mainly through them that connection between the Myxomycetes and the remainder of the fungi must be demonstrated, and, as already noted, there is sharp difference of opinion concerning this matter. If, as GÄUMANN states, they are unrelated to the remaining fungi, and the latter are regarded as having been derived from one or more algal groups, then the Myxomycetes cannot be included in the fungi. If, on the other hand, the Myxochytridiales and the Mycochytridiales are closely related, as FITZPATRICK and the majority of other mycologists believe, the inclusion of the Myxomycetes in the fungi, at least tentatively, offers no great difficulty.

Detailed discussion of the arguments for and against a monophyletic system of the fungi will be found in several of the works cited.

[1] In addition to the Myxomycetes, the following groups are mentioned in the report of the Special Committee on Nomenclature of Unicellular Organisms of the National Research Council as not distinctively either plants or animals: Spirochaetaceae, Chrysophyceae, Cryptophyceae, Dinophyceae, Volvocaceae, Euglenophyta, Chloromonadophyta.

It must be noted, however, that there are many chlorophylless organisms among the Thallophyta which are not fungi as the term is here applied. The bacteria may well be considered, at least in the light of our present information, as constituting an independent group of this nature. The saprobic and parasitic dinoflagellates are undoubtedly related to the holozoic and holophytic members of that group. The parasitic Florideae (**48, 52**) and the saprophytic diatoms (**43**) are to be regarded as members of the groups with which they are morphologically identified. Other less firmly established cases occur, as an example of which *Rhodochytrium*, believed by GRIGGS (**39**) to be a green alga, may be cited. These forms, with the possible exception of the last named, are no more to be regarded as fungi in a phyletic sense than are such angiosperm genera as *Monotropa* or *Corallorrhiza*. In this connection it might not be inappropriate to point out that the familiar phylogenetic trees which attempt to derive all living organisms from a single cell or a single primitive type are based upon an unwarranted assumption that life on the earth could have originated only once and in a single specifically determined form. From the zoological standpoint certain implications of this assumption are attacked by CLARK (**20**) in his stimulating argument for the historical independence of the animal phyla. CLARK'S case would have been stronger, however, had he carried his arguments to their logical conclusion, instead of referring repeatedly to "the primitive single cell."

Whatever may have been the origin of the fungi, it should be recognized that there is sufficient doubt of their frequently assumed algal ancestry to make it undesirable to refer to them in such a way as to imply that they are plants. Certainly, since the time when the nature of the mycelium and of its relation to the spore-bearing structures became known, there has never been any justification for the use of the term *plant* for the fructification of a fungus; and its extension to the entire organism (mycelium and fructifications) carries with it an implication which could be avoided without difficulty merely by substituting the word *fungus*. Again, whatever view be held regarding the nature of the fungi, the reference to the mycelium as a vegetative structure is most inappropriate. The terms *assimilative structure* and *assimilative phase* are certainly preferable if not

ideal; and an extension of the meaning of thallus and its derivatives might be made to apply to structures and processes where the word assimilative is unsatisfactory. Finally, the word *saprophyte* and its derivatives, implying that a fungus is a plant, can be replaced by saprobe (σαπρὸς+βίος), which is without such implication and has the advantage of being of as general application as the term parasite.

There are striking resemblances between the plasmodium of the Myxomycetes and the mycelium of many of the higher Phycomycetes, including many of the Saprolegniales or Mucorales, as well as conspicuous differences. They are alike in lacking a true cellular organization in their active assimilative and growing phases. Both consist of essentially homogeneous masses of protoplasm with numerous nuclei distributed throughout, sharing these characters with the Siphonales. All are what DOBELL (**27**) would call non-cellular organisms. If this is a legitimate basis for suggesting relationship between the Siphonales and the Phycomycetes, it is equally valid as an argument for suggesting relationship between the Phycomycetes and the Myxomycetes. In neither case is it decisive unless other characteristics can be homologized and the real nature of the differences which separate the groups evaluated. The arguments advanced by ATKINSON and others against a polyphyletic derivation of the fungi from various algal groups permit, as a reasonable hypothesis, the assumption of a monophyletic connection between the Phycomycetes and the Ascomycetes and Basidiomycetes.

I propose, therefore, to examine the differences which are held to indicate the existence of a wide gap between the Myxomycetes and the Phycomycetes, and to point out that a consideration of these differences will demonstrate that they are neither so complete nor so fundamental as to necessitate a greater separation of the two groups than is suggested by considering them to be separate classes of the same phylum. These differences are five in number: (1) The plasmodium is regarded as being formed by the fusion of numerous swarm cells or myxamoebae, while a mycelium is the direct and continuous outgrowth of a single spore. (2) The flagellum of the myxomycete swarm cell is always anterior; the attachment of the flagellum or flagella of the phycomycete zoospore is usually posterior

or lateral. (3) The nutrition of the mycelium is saprobic or parasitic, that of the plasmodium saprobic or holozoic. (4) The plasmodium lacks the permanent secreted membrane surrounding the mycelium. (5) The nuclei of the plasmodium are diploid, those of the mycelium haploid. These differences will be considered in order.

1. The belief that the plasmodium is formed as a result of the aggregation and fusion of numerous myxamoebae is based primarily on the work of CIENKOWSKI (**19**). DE BARY regarded this character as constituting the fundamental difference between the slime molds and the fungi. The studies of JAHN (**41**) and WILSON and CADMAN (**55**) have shown, however, that in the species they studied the plasmodium is initiated by the fusion in pairs of swarm cells or myxamoebae, functioning as gametes, with subsequent fusion of the nuclei. The diploid nucleus so formed proceeds to divide and thus inaugurates the multinucleate condition of the plasmodium. Unfused swarm cells or myxamoebae are often taken in, but only as food materials, and their nuclei and protoplasm are alike broken down and assimilated. It is true that small plasmodia may fuse to form a larger one, just as a large plasmodium may separate into two or more smaller ones; but as MASSEE (**47**) suggested, except for the fact that the process occurs more rapidly, it is not greatly different from the hyphal fusions known to occur in the mycelium of many different kinds of fungi (see, for example, BULLER **14**) or the mycelial separation due to the exhaustion of the older branches of a mycelium. According to this view, both the myxomycete plasmodium and the phycomycete mycelium develop from a uninucleate protoplast. SCHÜNEMANN (**51**), working with other species, found that a number of myxamoebae fused to form a small plasmodium with haploid nuclei, the nuclear fusion being delayed but evidently taking place at a relatively early stage, although it was not actually observed. He does not question the diploid condition of the nuclei in the older plasmodium. These results are not necessarily antagonistic, as a consideration of the phenomena accompanying the change from haploid to diploid mycelium in the Basidiomycetes as reported by BULLER (**14**) will demonstrate. A single spore of *Coprinus sterquilinus* may produce a completely coenocytic branched mycelium containing over two hundred haploid nuclei (**14**, fig. 52). The same

mycelium later becomes septate and the nuclei are associated as dicaryons. If, instead of a single spore, numerous spores germinate simultaneously, the hyphae so produced fuse indiscriminately, producing a single vigorous mycelium able to dominate the substratum (**14**, p. 163). It is not suggested, of course, that the Myxomycetes and the Basidiomycetes are closely related. The differences between a myxomycete plasmodium and basidiomycete mycelium are apparent and need not be minimized; but in considering these differences, it is important not to overlook certain striking and suggestive similarities.

2. The anterior flagellum in the swarm cell of the Myxomycetes is extremely constant. The only variation occurring is the occasional appearance of swarm cells, otherwise normal, with two flagella (GILBERT **35**, HOWARD **40**). Reports of swarm cells with a flagellum at each end are shown by GILBERT to be due to contamination of cultures by a protozoan. On the other hand, the zoospores of the Phycomycetes, and particularly of the Myxochytridiales, vary greatly so far as the insertion of the flagella is concerned, as already emphasized by GRIGGS (**38**). The number may be either one or two but is constant in a given genus. A few cases have been recorded in which the swarm cell has a single flagellum directed forward. Such are *Reesia* (FISCH **29**) and *Sphaerita* (DANGEARD **26**), both genera being included by recent writers among what are here referred to as the Myxochytridiales. In *Saprolegnia* and some of its relatives the zoospores are provided with two apical flagella during the first swarming period (**23**). In view of the very great variability of this character among the Phycomycetes, and its at least occasional agreement with the situation in the Myxomycetes, it seems unwise to regard it as a matter of primary taxonomic significance. DANGEARD regards it as important but subordinate to other characters (**26**, p. 242).

3. The nutrition of the Phycomycetes is saprobic or parasitic. In the Myxomycetes, swarm cells, myxamoebae and plasmodia are known to ingest solid particles and living cells and to digest them. This capacity, first noted by DE BARY, has been repeatedly confirmed by other students, among whom may be cited JAHN, GILBERT (**36**), and SKUPIEŃSKI (**53**).This type of nutrition is clearly holozoic. On

the other hand, it has long been believed that the principal way in which food is taken in is in the form of dissolved organic materials, and this is verified by the capacity of plasmodia to grow in culture media from which all solid particles have been filtered out. The destruction of the hymenial surface of fleshy fungi, as noted by LISTER (**45**) and a number of observers since his time, needs further investigation, as it is not certain whether the hyphae and the hymenial elements are ingested and then digested, or whether they are dissolved by enzymatic action and the products absorbed. The evidence at the present time indicates that nutrition may be either saprobic or holozoic, and, at least in some cases, both at the same time. The subordinate value of nutritive relations in determining taxonomic position is well illustrated by the dinoflagellates, in which group the same genus, for example *Gymnodinium*, may contain holozoic, holophytic, and saprobic species (**44**). Furthermore, the differences between parasitic nutrition as found in the Myxochytridiales and holozoic nutrition are no greater than those between the saprobic and parasitic habits, although it is often assumed that the latter must have arisen from the former. The differences in nutrition between the Myxomycetes and the other fungi are not fundamental, therefore, but are determined by the lack of a firm membrane in the former group, which has permitted it to retain a more generalized type of assimilation while becoming highly specialized in other ways.

4. The myxomycete plasmodium is commonly spoken of as "naked." This, however, is only as compared with the firm envelope surrounding the protoplasm in the bulk of the Phycomycetes and in the higher fungi. As already pointed out, the Myxochytridiales are intermediate in this respect. For three-quarters of a century it has been recognized that the plasmodium is not homogeneous. DE BARY in his first paper (**3**) states that the surface is surrounded by a gelatinous membrane, "deren weichheit es möglich macht, dass sie den Bewegungen folgt oder dass sie an dem Substrat theilweise Kleben bleibt, während der Sarcodestrang fortkriecht und (wahrscheinlich) neue Hüllhaut ausscheidet." The importance of this observation must be emphasized, since CIENKOWSKI (**19**) denied this and his account of the nature of the plasmodium as well as of its origin has

been generally accepted. Nevertheless careful examination, either of living or fixed material, will show that the inner, granular portion of the plasmodium is surrounded by a hyaline outer layer of firmer texture, just as stated by DE BARY, very delicate at the advancing margin but thicker and firmer in the veinlike strands farther back. If this outer layer is broken, the more liquid material in the interior may flow out, quickly forming a new outer layer. MASSEE long ago pointed out the resemblances between such strands and the mycelium of a Mucor (**47**). It has also often been noted that as the plasmodium crawls about on its substratum it leaves behind a reticulate trail of "waste material." What seems to have escaped observation, however, or at least adequate emphasis, is the fact that this reticulum is composed, not of a mere deposit upon the substratum, but of very soft and gelatinous collapsed hyaline tubes within or upon which the waste material is left. These tubes, as found in a vigorously growing species such as *Physarum polycephalum*, suggest the empty tubes left behind by the rapidly growing branches of some of the Phycomycetes, for example *Conidiobolus*. They differ in their much larger size, their gelatinous texture, and the absence of septa. They are sometimes firm enough, if handled with care, to be lifted from the substratum with a needle; but removal is easier if they are killed and hardened in acetic alcohol or other fixing agent. The walls are thicker at the sides than at the top, and shortly after the protoplasmic core has been withdrawn the tube collapses, taking on the appearance of a gelatinous trough in which the protoplasm has moved. The walls are very soft and are frequently pierced by protoplasmic branches. Nevertheless the membrane is present, and appears to differ in degree, rather than in kind, from that surrounding the protoplasm of a typical Phycomycete.

5. There remains to be considered only the nuclear condition of the plasmodium. The investigations of JAHN (**41**), WILSON and CADMAN (**55**), CAYLEY (**18**), and HOWARD (**40**) seem to demonstrate that the nuclei of the plasmodium are diploid. It seems equally clear that the nuclei of the mycelium in the higher Phycomycetes are haploid (**34**). This difference must be regarded as of considerable importance. The situation among the Myxochytridiales is for the most part obscure, but such evidence as we have would suggest that there

is but one diploid nucleus in the life cycle. The nuclei of the so-called plasmodium in the Plasmodiophoraceae may be diploid; but these organisms, in their nuclear behavior and in other respects, are so aberrant that their position must be regarded as dubious (COOK **24**) and it would be hazardous to base conclusions on supposed affinity with them. It remains true, however, that nuclear differences between the Phycomycetes and the Myxomycetes are no greater than those between the Phycomycetes on the one hand and the Ascomycetes and Basidiomycetes on the other. That is to say, they are no greater than might be expected between two classes which, originating from the same source, have followed widely divergent lines of development.

To summarize briefly: it is pointed out that the supposed origin of the plasmodium by the fusion of numerous myxamoebae is not a constant feature in the Myxomycetes, and where it does occur the process is not so radically different from the process of mycelial development in the other fungi as has been assumed. It is suggested that in view of the great variation noted among the zoospores of the Archimycetes and Oomycetes, the character of the myxomycete swarm cell cannot be regarded as indicating a total lack of relationship between the Myxomycetes and the Phycomycetes. The holozoic and saprobic nutrition of the Myxomycetes may be regarded as a generalized character retained by reason of the peculiar nature of the plasmodial membrane. This is very soft and gelatinous, but nevertheless resembles that of the Phycomycetes in that it takes the form of hollow tubes through which the protoplasm moves. The nuclear condition of the slime molds differs no more from that of the Phycomycetes than does that of the latter from that of the Ascomycetes and Basidiomycetes. All of these differences find adequate taxonomic expression if the slime molds are regarded as a distinct class of the fungi, coordinate with the other recognized classes.

The views here advanced are not presented as original. This paper will have served its purpose if it calls attention to some old but neglected observations which, when fitted together, seem to provide a truer picture of the position of the fungi among living organisms, and of the interrelationships within the group, than is to be had from the conclusions more generally accepted.

Summary

1. The Myxomycetes have been regarded both as fungi and as animals. If the fungi are thought of as descended from green algae either as a monophyletic or polyphyletic group, the Myxomycetes are excluded. If, however, the fungi are regarded as a monophyletic group descended from colorless flagellates, the Myxomycetes form a natural class, the lowest of four, the others being the Phycomycetes, the Ascomycetes, and the Basidiomycetes. It is suggested that the fungi be regarded as a phylum which has not definitely developed into either plants or animals, but may be grouped with the former as a matter of convenience, and in accordance with custom. It is pointed out that the familiar assumption that all living organisms are descended from a single primitive cell is unwarranted and less in accord with what we know concerning living organisms than the assumption that life may have originated more than once and in different forms.

2. It is suggested that the words fungus, assimilative and saprobe, and appropriate derivatives, be used instead of plant, vegetative, and saprophyte, in referring to fungi.

3. The differences between the Myxomycetes and the Phycomycetes which are usually held to indicate a sharp separation of the two groups are considered, and it is maintained that these differences are neither so absolute nor so fundamental as is ordinarily believed, and may be recognized adequately by regarding them as distinct classes of a single phylum.

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ECOLOGICAL ASPECTS OF TRANSPIRATION

I. PIKE'S PEAK REGION: CLIMATIC ASPECTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 437

CHARLES J. WHITFIELD

(WITH EIGHT FIGURES)

Introduction

During the past three years, transpirational and instrumental studies have been conducted in the principal vegetational climaxes of the Pike's Peak region, Colorado, and Santa Barbara County, California. These regions are well suited for studies of this type because in each of them there are several climaxes in close proximity to one another. In the Pike's Peak region, in a distance a little more than seven miles by airline, six climaxes are represented (CLEMENTS 1). Since the chaparral and woodland climaxes are limited in extent, little or no experimental work was done in these two associations. The remaining four climaxes, plains, montane forest, subalpine forest, and alpine tundra, are the conspicuous ones of the region.

The plains, with *Bouteloua gracilis*, *Stipa comata*, and *Sporobolus cryptandrus* as the conspicuous dominants, are characterized by high temperatures, saturation deficit, evaporation, and wind; and by low relative humidity, holard, and rainfall. *Pseudostuga mucronata* and *Pinus ponderosa* are the important dominants of the montane zone, which is characterized by higher relative humidity, holard, and rainfall; and by lower temperatures, saturation deficit, evaporation, and wind. The dominants of the subalpine association are *Picea engelmanni* and *Pinus aristata*. This zone has higher relative humidity, rainfall, and wind than the montane zone, and lower temperatures and saturation deficit. The climax species of the alpine tundra are *Elyna bellardi* and *Carex rupestris*. Here are the highest relative humidity, holard, wind velocity, and rainfall, and the lowest temperatures and saturation deficit.

The ecology of this region has been discussed in two previous papers which deal with vegetational and instrumental aspects of the

different climaxes (**7, 8**). The objects of the present study are the measurement of the water loss in relation to the physical factors in as many climaxes as possible, and the establishment of correlations between factor and function. The materials for this paper have been drawn from the projects now being conducted in this region by the Carnegie Institution of Washington under the direction of Dr. F. E. CLEMENTS.

Methods

Transpiration of native and control plants (sunflower, corn, wheat) was measured in the different climaxes by means of the phytometer or gravimetric method; environmental data were obtained by using batteries of instruments. The historical aspects of the phytometer method have been discussed by CLEMENTS and GOLDSMITH (**2**) and MAXIMOV (**5**). In this paper the term phytometer is employed to designate any plant or group of plants used for functional purposes, and implies the use of the gravimetric method (**2**). The methods used here, although they agree in principle, differ in detail from those employed by BRIGGS and SHANTZ, WEAVER, and CLEMENTS and GOLDSMITH.

EQUIPMENT.—Unless otherwise stated, cylinders 9 inches tall by 5.5 inches wide were used. These were made of 28-gauge galvanized iron, and waterproofed by soldering the bottoms and sides. The containers were equipped with slightly tapering lids with a 2-inch opening. Both the lid and the cylinder were provided with a 0.75 inch crimp to permit a closer fit, and the lid was further sealed by plumber's tape or an equal mixture of beeswax and paraffin. Near the bottom of each container a bicycle valve-stem, with the core removed, was inserted for aeration. For watering purposes a glass tube, 0.5 inch wide and approximately 11 inches long, was inserted in each container through the opening of the lid. A rubber stopper was put in the glass tube to prevent evaporation. In all cases, a half-inch of coarse gravel was placed in the bottom of the container, level with the lower end of the glass tube. With the water equally distributed in the gravel, movement into the soil took place more evenly.

PLANTS, STANDARD AND NATIVE.—When sunflowers, wheat, or corn, the controls or standards, were used, the following procedure was employed. In order to have enough material for selective pur-

poses, approximately twice as many plants were grown as were needed for the various stations. First the gravel and the glass tubes were placed in the desired number of containers. Next the latter were filled with soil previously screened and of good tilth. In order to pack the soil, the cans were lifted approximately 6 inches from the ground and allowed to fall several times during the filling. When the containers were full, with lids sealed in place, the seeds were planted just below the soil surface. They were then watered and covered to prevent drying, and allowed to develop to the four-leaf stage.

After the plants had reached this stage they were watered, sealed, aerated, and standardized. By means of a burette, the same amount of water was added for each of the plants. Each container was aerated by attaching to it a water aspirator, or suction pump, fastened to an ordinary garden hose. Gas analyses showed that aerating each container for 4 minutes was sufficient. The results of a 4-minute test were: before aerating, oxygen, 18.71 per cent; carbon dioxide, 2.09 per cent; after aerating, oxygen, 20.95 per cent; carbon dioxide, 0.48 per cent.

For standardization purposes, a temporary seal of very fine sand or a light coat of paraffin was sufficient to prevent evaporation. Leaf areas were determined, the plants were weighed, and then placed in a greenhouse on a revolving table which rotated once every 10 minutes. After 2 days the plants were remeasured, reweighed, and classified according to their losses. Plants which transpired the same amount on the revolving table were distributed among the various climaxes or stations, the same number of plants being sent to each station. In this way not only the individual plants but also the batteries at each station were equivalent.

After standardization, the plants were brought to their original weight by watering through the tubes. They were then aerated and permanently sealed. In many cases assistants were located at each station to protect the phytometers from rain, so that the permanent sealing was chiefly a matter of prevention of evaporation. When the plants were left unprotected, however, it was necessary to seal them, not only to prevent evaporation but also to prevent rain from entering. After a number of years of experimentation in different habitats, it became evident that each climax required a particular seal.

At Santa Barbara, where the experiments can be conducted between rains, all that is necessary is a fine sand over the soil, with a piece of oilcloth over the top of the can to prevent the wind from blowing the sand. On the Colorado plains, with both high temperatures and rains, non-absorbent cotton and tinfoil in the form of miniature umbrellas were placed around the stem. The cotton was used to permit expansion by growth, as well as to prevent the warm paraffin from coming in contact with the stem. The tinfoil umbrellas helped deflect the rain which might otherwise run down the stems of the plants. The soil was covered with a mixture of equal parts of beeswax and paraffin. The gap left between the seal and the container was filled in most cases by melting the outer edge of the former with a hot rod, and in other cases by plasticine. This seal was further protected by rubber sheeting or oilcloth.

Finally the phytometers were again measured and weighed, and then placed in typical habitats. A typical battery for each station consisted of eight to ten plants. The plants were taken to the various habitats by Cog train and automobile in Colorado, and by automobile in California. In all cases, unless otherwise mentioned, the containers were sunk in the ground so that the plants would be at the same level as the native vegetation; this had the further advantage of keeping the soil temperature of the phytometers approximately the same as that of the surrounding habitat. The average length of time for most of the series was 2–4 days. The plants were remeasured and reweighed at the end of the experiment, and returned to the laboratory for restandardization. Sometimes, if the plants were in good condition, the experiments were continued for another period of 2–4 days.

Losses were figured on the basis of either leaf area or standardization. In figuring leaf areas of sunflowers, the method developed by CLEMENTS and GOLDSMITH (**2**) was used. Briefly, the leaf area of sunflower leaves in square centimeters was found by multiplying the product of the extreme dimensions measured in millimeters by the factor 0.013. For wheat and corn, the length and width of each blade were measured. When native plants were used, this procedure was followed with the exception that the containers were filled with native soil, and the transplants allowed time for readjustment.

SHORT-PERIOD PHYTOMETERS.—In all the experiments, short-period phytometers were employed. Small cylinders of the size already mentioned are more easily distributed, weighed, and protected in the field than are those of larger dimensions. With small containers it is also possible to have a greater number of plants in each habitat, which is a very desirable factor. By this method the initial responses of the phytometers to the various habitats are measured. Moreover, when plants like sunflowers are used, they are not left in small containers long enough to cause unnatural root development, which influences the functional responses.

The results of experiments conducted with short-period phytometers and potometers at different stations showed that the functional and stomatal responses of the potometers did not coincide with those of the phytometers. These conclusions led to the exclusive use of short-period phytometers in this work.

Results

In order to measure the environmental factors, batteries of instruments were placed with the phytometers in the various habitats. The battery, together with the simple instruments, consisted of a hygrothermograph, cog psychrometer, thermometer, evaporimeter, black and white atmometers, anemometer, and rain-gauge. In Colorado, soil thermographs were also used.

FIRST SERIES.—Two species, *Helianthus annuus* and *Triticum sativum*, were used as phytometers in the initial series. Since a revolving table was not available in this case, the plants were placed in a greenhouse and each phytometer shifted every hour during the day. General sky conditions during the experiment were similar at the three stations, with the mornings clear and the late afternoons usually cloudy. A slight rain was reported throughout in the afternoon of Friday, June 28.

The average leaf area of the ten plants of *Triticum sativum* was 24.85 sq. cm. at the plains station, 24.25 sq. cm. at the montane station, and 26.57 at the alpine station. The average leaf area of *Helianthus annuus* at the three stations was 75.08, 72.43, and 68.48 sq. cm. respectively.

The factor data were secured and computed according to methods

described in a previous paper (**7**). The experiment was started at 6 P.M., June 25, and closed at 6 P.M., June 28, 1929. Readings were made at 6 P.M. and 6 A.M. throughout the series. The day and night readings thus secured were averaged and added to get the daily averages shown in figure 1. All are daily averages except the relative humidity, which is for the day only.

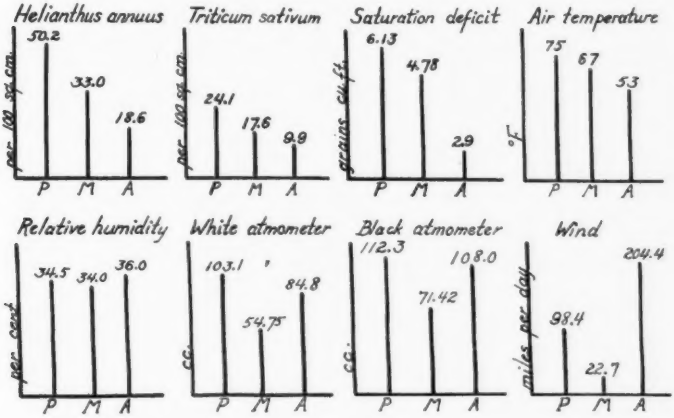


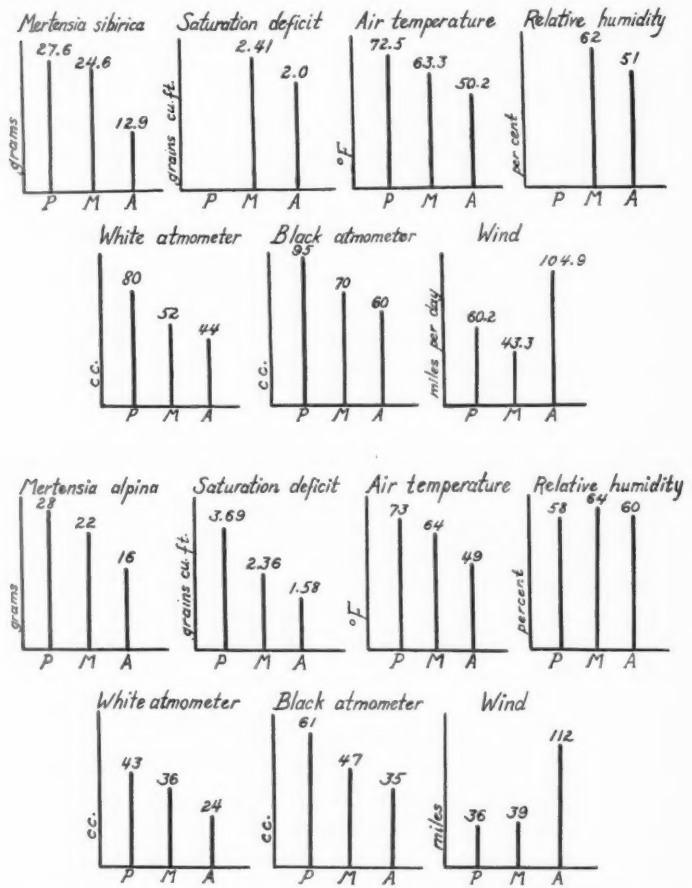
FIG. 1.—First series, June 25–28, 1929: *P*, plains, *M*, montane; *A*, alpine

SECOND SERIES.—In this experiment specimens of a native species, *Mertensia sibirica*, were used as phytometers. This species was chosen because of its value in the transplant experiments, large leaf surface, and wide distribution. During the series no rain fell at the plains, 0.3 inch fell at the montane, and 0.07 inch fell at the alpine station. The plants in all cases were protected from rain. Saturation deficits based on readings of air temperatures and relative humidities taken at 6 A.M., 12 noon, and 4:30 P.M. on July 5 were: plains, 8.47; montane, 4.34; and alpine, 2.42 grains per cu. ft.

The series was conducted during the period July 3–7, 1930. Readings were made at 6 A.M. and 6 P.M. (fig. 2).

THIRD SERIES.—*Mertensia alpina*, an alpine species, was employed in this series. A smaller container, 5 inches tall and 5.5 inches wide, was used. The series was conducted at the three climatic stations

during the period July 9–12, 1930. Weighings were made at 6 P.M. (fig. 3).



FIGS. 2, 3.—Fig. 2 (above), second series, July 3–7, 1930; daily averages. Fig. 3 (below), third series, July 9–12, 1930; daily averages.

FOURTH SERIES.—Three species, *Helianthus annuus*, *Mertensia sibirica*, and *M. alpina* were used as phytometers at six stations: plains, woodland, montane, subalpine, alpine, and summit. *Helian-*

thus was distributed to all stations; *Mertensia sibirica* to the plains, montane, subalpine, and alpine stations; and *M. alpina* only to the

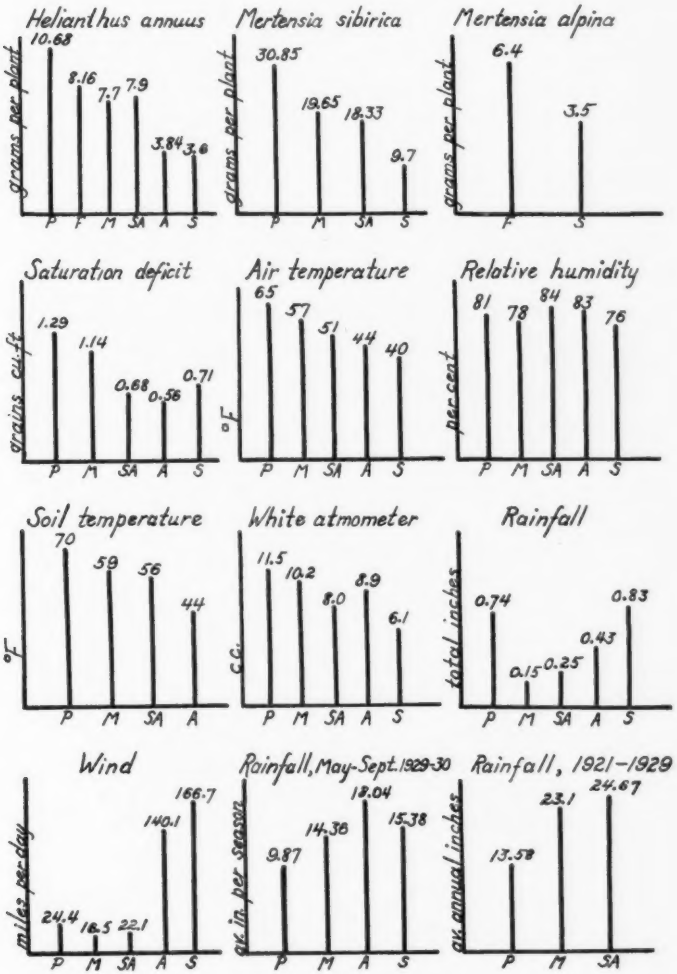


FIG. 4.—Fourth series, July 28–31, 1930: *P*, plains; *F*, foothills; *M*, montane; *S*, subalpine; *A*, alpine; *S*, summit.

foothills and summit. Weather conditions during this experiment were adverse. Rain was reported at all stations, but it was particu-

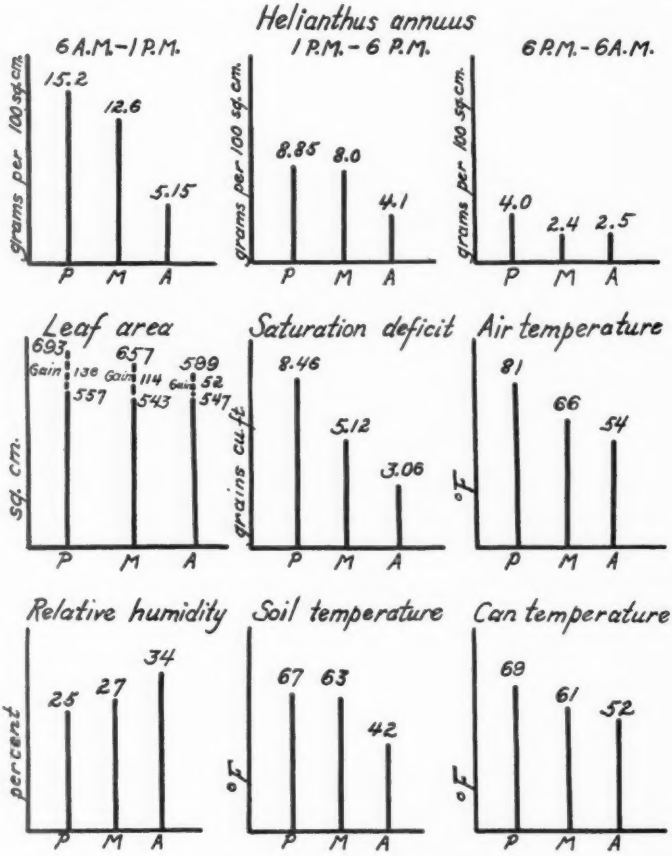


FIG. 5.—Fifth series, July 18–20, 1931

larly heavy at the plains and summit. In general, however, the climatic environmental relationships of the various stations were maintained.

The experiment was conducted from July 28 to 31, 1930. Readings were made at 5 P.M. daily (fig. 4).

FIFTH SERIES.—*Helianthus annuus* was used in this series. The phytometers were placed in lath-houses at each station. During the

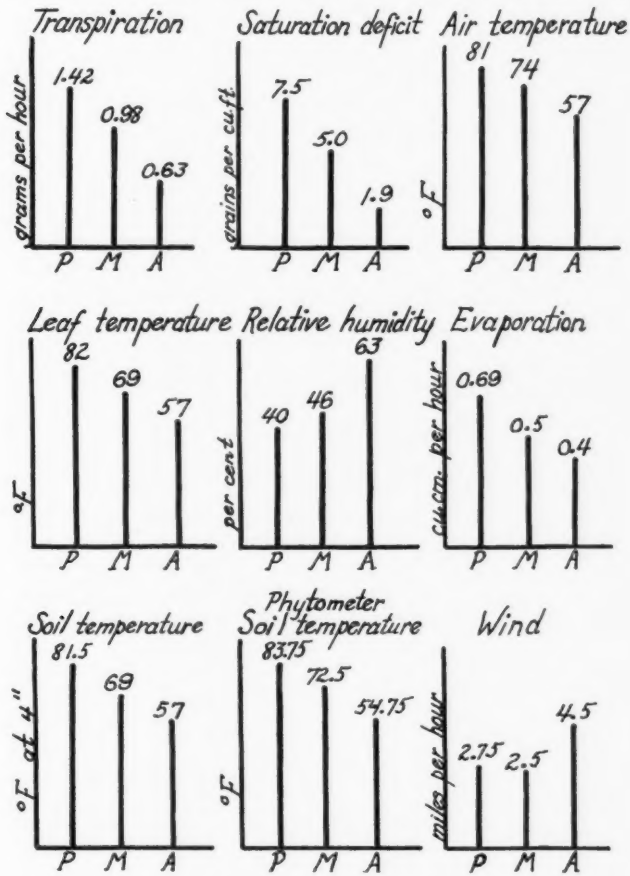


FIG. 6.—Sixth series, July 27–30, 1931 (see table I)

experiment light readings were taken in the lath-houses. Averages of three readings, made at 9 A.M., 12 noon, and 3 P.M., were as follows: plains, 19.5; montane, 18.4; and alpine, 17.2 per cent. Hand photometers were used to measure the light.

TABLE I

TRANSPIRATIONAL AND INSTRUMENTAL DATA, SIXTH SERIES (CF. FIGURE 6)

	TIME																		
	7/27 6 P.M.	7/28 6 A.M.	8 A.M.	10 A.M.	12 N.	2 P.M.	4 P.M.	6 P.M.	8 P.M.	12 MT.	7/29 4 A.M.	6 A.M.	8 A.M.	10 A.M.	12 N.	2 P.M.	4 P.M.	6 P.M.	7/30 6 A.M.
Transpiration per 100 sq. cm.																			
P	.....	1.8	3.7	5.9	7.6	5.8	5.3	2.6	0.8	0.7	0.4	1.0	3.1	5.3	6.4	7.4	8.3	4.9	2.8
M	.....	1.8	3.6	5.5	7.4	4.3	1.5	1.8	0.4	0.1	0.1	0.2	2.9	4.9	6.1	6.9	6.2	3.6	1.9
A	.....	1.8	2.0	3.7	4.6	1.9	1.2	1.2	0.6	0.5	0.6	0.3	1.5	3.0	4.0	4.1	3.4	2.2	1.3
Air temperature																			
P	80	65	86	92	94	87	80	76	71	63	60	68	74	80	90	85	91	85	61
M	70	65	79	85	88	78	70	68	62	56	56	57	70	75	75	79	77	69	60
A	56	53	58	67	68	58	58	55	51	46	46	46	53	60	61	61	60	56	47
Leaf temperature																			
P	88	68	85	90	92	82	83	86	72	66	63	70	72	80	86	80	88	85	81
M	68	61	75	82	83	72	64	61	55	52	52	54	69	76	73	79	77	59	56
A	58	52	55	64	62	57	56	53	52	46	.....	48	58	60	60	59	57	55	46
Relative humidity																			
P	30	57	33	27	21	32	38	49	52	64	67	63	60	54	38	27	15	17	64
M	49	52	41	31	28	35	52	54	54	56	63	61	55	53	43	33	28	43	57
A	73	83	70	62	62	74	69	59	73	87	75	81	65	56	54	52	48	64	81
Saturation deficit																			
P	7.65	2.92	8.53	11.45	13.14	9.20	6.78	4.92	3.96	2.29	1.90	2.77	3.63	5.03	8.90	9.30	12.95	10.57	2.14
M	4.07	3.17	6.25	8.52	10.00	6.68	3.83	3.44	2.82	2.21	1.86	2.02	3.59	4.40	5.34	7.10	7.18	4.41	2.47
A	1.36	0.74	1.61	2.84	2.84	1.30	1.61	2.06	1.27	0.46	0.89	0.70	1.58	2.36	2.73	3.15	2.89	1.81	0.70
Soil temperature																			
P	91	77	76	80	83	86	85	85	83	78	75	74	74	78	84	90	88	86	74
M	73	66	65	66	69	72	73	72	72	67	65	64	63	65	68	72	75	75	65
A	60	51	60	53	60	62	62	59	56	53	50	49	49	52	58	64	66	66	50
Phytometer soil temperature																			
P	94	79	76	80	87	90	90	87	84	78	75	73	73	77	83	89	93	94	73
M	80	66	65	70	75	82	77	74	71	65	63	61	62	67	74	80	81	82	64
A	77	51	52	54	59	62	60	58	56	53	49	48	49	53	57	53	66	64	51
Piché evaporimeter																			
P	.....	3.58	1.67	3.60	4.0	2.3	1.80	1.0	0.8	1.4	0.6	0.4	1.7	2.6	1.62	2.68	3.3	2.9	3.3
M	.....	3.6	1.05	2.05	2.9	1.9	0.8	0.8	0.5	1.2	1.1	0.6	0.8	1.2	1.9	2.50	2.1	1.7	3.3
A	.....	2.65	1.25	1.50	2.2	1.14	0.5	0.75	0.75	1.0	1.1	0.5	0.8	1.2	1.8	1.80	1.6	1.2	2.1
Anemometer																			
P	.....	16.2	3.59	16.2	11.1	6.2	4.4	0.71	2.7	13.7	1.9	2.4	22.2	20.4	9.5	6.9	9.2	7.6	7.9
M	.....	26.9	3.1	4.5	9.6	5.8	5.7	4.2	4.7	8.9	10.2	6.0	3.9	5.7	6.9	8.7	6.6	4.9	27.0
A	.....	40.4	12.8	11.6	14.9	13.3	8.8	7.5	17.9	5.4	15.8	9.2	8.1	6.8	10.8	11.0	11.7	7.3	41.6

The series was started at 1 P.M., June 18, and closed at 1 P.M., June 20, 1931. Weighings were made at 6 A.M., 1 P.M., and 6 P.M. (fig. 5).

SIXTH SERIES.—Plants of *Helianthus annuus* were used as phytometers for the series conducted at the plains, montane, and alpine stations (fig. 6 and table I). General sky conditions at the three stations were similar, all three having approximately the same amount of sunshine and cloudiness. The first day was clear until noon and cloudy all afternoon. During the second day weather conditions were more or less intermittent up to three o'clock and then clear. Hand-photometer readings taken every hour at the three stations

TABLE II

STOMATAL RELATIONS OF PHYTOMETERS, SIXTH SERIES. KEY: 0=closed; ½=nearly closed; 1= ⅕ open; 2=⅖ open; 3=⅗ or more open

	TIME																	
	6 A.M.	8 A.M.	10 A.M.	12 M.	2 P.M.	4 P.M.	6 P.M.	8 P.M.	12 MT.	4 A.M.	6 A.M.	8 A.M.	10 A.M.	12 M.	2 P.M.	4 P.M.	6 P.M.	6 A.M.
Plains	0	1–0	1–0	1	2	2–3	2	1–2	1	2	1–2	2	3	1	3	1	1	1
Montane	1–2	2–3	1–2	2	1	0–1	½	0–½	0	0	0–½	1	1	0–½	½	0–½	½	0–1
Alpine	1–2	2	2	2	1–2	0–1	½	1–2	0–1	2	0–½	1	1–2	1–2	1	½	½	½

showed the following averages: plains 62.3, montane 46.9, and alpine 52.7 per cent. Averaged evaporimeter readings made simultaneously at the three stations were: plains 6.55, montane 4.53, and alpine 3.27 gr. per 100 cm.² per hour. These results are in general accord with those secured by the Piché evaporimeter. Leaf areas were taken at the beginning and end of the experiment. Greatest growth took place at the plains, with the least at the alpine station, the increases being 42, 38, and 32 sq. cm. per plant respectively.

The condition of the stomata with reference to the degree of opening was also considered. Stomatal strips were made and examined from plants used only for this purpose.

The stomatal relations are shown in table II.

The experiment was started July 27, 1931, at 6 P.M. and closed at 6 A.M., July 30. Weighings and stomatal strippings were made at 2-hour intervals from 6 A.M. to 6 P.M., and at 8 P.M., 12 midnight, and

4 A.M. during the night of July 28–29. Instrumental records were made hourly between 6 A.M. and 6 P.M., and at 8 P.M., 12 midnight, and 4 A.M. during the night of July 28–29.

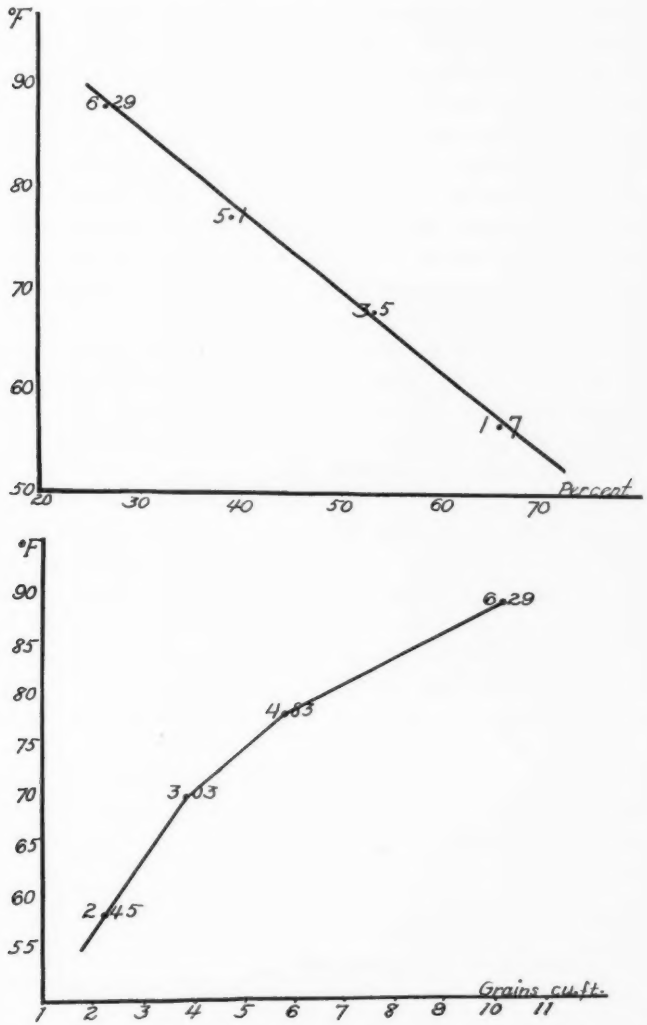
Discussion

Helianthus annuus, *Mertensia sibirica*, *M. alpina*, *Triticum sativum*, and *Chamaenerion angustifolium* were used as phytometers in the various climatic formations of the Pike's Peak region, Colorado. Eight different climatic series were conducted to record the transpiration and the climatic conditions, and to study the relations between them in the principal formations.

In all cases transpiration decreased with increased altitude. This was true for all species used in the different series. Of the various climatic conditions measured, evaporation, soil and air temperatures, and saturation deficits decreased with increased altitude. On the other hand, the approximate values of the maximum intensity of radiant energy on a clear day were 1.48, 1.58, and 1.64 cal. per cm.^{2} per min. for plains, montane, and alpine respectively, showing increased values with increased altitude. However, the total energy over a season might reverse these readings because of the more cloudy conditions in the alpine region. No data are at present available on this problem. Relative humidities, rainfall, and holard are usually greater at the higher elevations. Wind is constantly highest in the alpine region, with the plains next, and the montane zone ordinarily the lowest.

Few exact studies have been made in the field of the relationship between transpiration and the main climatic factors. In the present studies, close correlations have been secured with isolated factors as well as combinations of some of the principal climatic factors.

A close relationship exists between transpiration, air temperature, and relative humidity, and between transpiration and a combination of these two factors (fig. 7). The average air temperature, relative humidities, and transpiration of the natural groups approximates a straight line. This relationship is strengthened by the fact that the average air temperatures, relative humidities, and transpiration at the main climatic stations indicate a straight line. While the results approximate a straight line between transpiration, air temperature,



FIGS. 7, 8.—Fig. 7 (above), temperature-relative humidity-transpiration. Fig. 8 (below), temperature-saturation deficit-transpiration.

and relative humidity, such a correlation does not exist between transpiration, saturation deficit (fig. 8), and evaporation. The transpirational points which fall naturally on an approximately straight line with air temperature and relative humidity do not do so with saturation deficit and evaporation. These results show that the two curves of transpiration and saturation deficit do not run parallel. In addition, the mathematical ratios of transpiration and saturation deficit are irregular. The fact that the points do not approximate a straight line, as well as the irregularity of the ratios, indicates that saturation deficit does not bear so close a relation to transpiration as do air temperature and relative humidity.

In comparing transpiration and evaporation per 100 cm.^{2} per hour, the water loss of the improved evaporimeter (**6**) was approximately five times that of the phytometers. The ratios between the two were regular for the plains and alpine stations, but were irregular for the montane.

Observations made on sky conditions during the various series indicate a relation between transpiration and radiant energy. During the first day of the sixth series the sky was clear until noon and cloudy in the afternoon; during the second day weather conditions were more or less variable up to 3 o'clock and then clear until sunset. Transpiration showed an increase until noon of the first day and then decreased; while during the second day transpiration rose in steps, reaching the maximum about 3 P.M., then dropping with the other factors.

The results of growth measurements made during the various series show that the greatest increase took place at the plains station, the smallest at the alpine station. The ratios between transpiration and growth at the plains, montane, and alpine stations were 0.207, 0.202, and 0.226 respectively, indicating a close relationship between these two functions.

These results indicate relations between factor, function, and vegetation. Climatically the conditions at the plains station are the best for growth, but due to low soil moistures the vegetation is mixed prairie. The montane zone, although having a shorter growing season than the plains, is not limited by any other climatic or edaphic factor. In consequence the climatic vegetation is forest rather than

grassland. The alpine tundra shows the least xeric tendencies of all by having low transpiration, evaporation, and saturation deficits, and high humidities and holard. In spite of these mesophytic characteristics, however, the alpine vegetation is composed of dwarf grasses and sedges. This is probably due to the reduced air pressure which expresses itself most strongly in the low air and soil temperatures. The temperatures often become so low during the short growing season that the availability of water is limited. It is in such times of stress that the xeromorphic structures of the alpine plants become useful by limiting to a great extent the water loss.

Summary

1. A comparison of the plains, montane, and alpine climates of the Pike's Peak region showed that: (a) air and soil temperatures, saturation deficit, and evaporation decrease with increased altitude; (b) radiant energy, relative humidities, rainfall, and holard are usually greater at higher elevations; (c) wind movement is constantly highest in the alpine region, with the plains next, and the montane zone ordinarily the lowest.

2. A comparison of the functional responses of plants in these three climates showed: (a) a decrease of transpiration with increased altitude; (b) a decrease of growth rate with increased altitude.

3. The transpiration curve showed a closer correlation with the air temperature and the relative humidity curves than with any of the other factors measured.

4. Evaporation was approximately five times as great as transpiration in the three regions.

5. Vegetation does not respond to any one factor, but rather to all of the climatic conditions.

The writer wishes to express his obligation to the Carnegie Institution, and especially to Dr. F. E. CLEMENTS and Dr. H. C. COWLES, for much helpful advice and criticism.

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OSMOTIC PRESSURE OF LEAVES OF PINUS SCOPULORUM, AND CERTAIN ENVIRONMENTAL FACTORS[1]

J. Fisher Stanfield

(WITH THREE FIGURES)

Introduction

In the past, many workers have advanced the theory that cell-sap concentration of leaves is inversely proportional to the amount of water in the soil, and have taken osmotic pressure of leaves as a basis for this contention. Later writers have differed, and there seems to be a marked tendency to disregard or disclaim the theory that the osmotic pressure of the leaves of any species is a valid criterion of the ecological requirements of the plant.

Tests were conducted with *Pinus scopulorum* at Boulder, Colorado, over a period of 16 months, to note the relationship of the pressure of the needles to the environmental complex. Advantage was taken of differences in habitats found in this vicinity and the presence of *P. scopulorum* for year-around determinations.

Only recently has the pressure of the cell sap been studied to any degree in connection with the habitat factors. The work of Korstian (**12**) and Gail and Cone (**5**) is of particular interest in regard to *Pinus*. The procedures used in determinations given in this paper have been influenced by contributions of others working in this field (**7, 8, 11, 14, 15, 16**).

Experimental methods

Two-year-old needles were used. Collections were usually made between 3 and 5 P.M. at various intervals over a period of 16 months on Reservoir Hill, which is located one mile south of Boulder, Colorado. The size of the trees is given in the results for the various series of tests.

After collection, the needles were placed in pint Mason jars and

[1] Contribution no. 41 from the Biological Laboratories of Knox College and the Botanical Laboratories of the University of Colorado.

frozen overnight at a temperature of $-10°$ F. in the hardening room of a local creamery. As little time as possible was lost between the collection and freezing. After thawing, the needles were cut into small pieces and pressed in a specially constructed press, as described by MEYER (**14**), with an applied pressure of approximately 5000 pounds to the square inch. Sap density determinations were made by means of the standard freezing-point method. The osmotic pressure of the sap was calculated from the observed depression of the freezing point by using the formula suggested by HARRIS and GORTNER (**7**).

The rate of evaporation was determined by means of standardized Livingston atmometers, and the average of results from three stations is given in the tables. The atmometers were placed approximately 4 feet from the ground, as the trees tested in this series of determinations were from 4 to 6 feet in height.

Soil temperatures were taken at a depth of 1 foot and at a distance of about 3 feet from the tree. Records of air temperatures were obtained from the local government observer and the mean temperatures calculated for the cited periods. The soil for moisture determinations was taken at a depth of 1 foot and about 3 feet from the trees tested. Samples were taken from opposite sides of the tree and the average of the two tests is given. The amount of moisture was computed on the basis of the oven-dry weight of the soil.

The hygroscopic coefficient was determined by the method given by WEAVER and CLEMENTS (**19**). A mixture of all the soils tested for moisture content was used, and the duplicate tests which were run gave a very close check. The percentage of hygroscopic water was computed on the basis of the weight of oven-dried soil.

Experimental results

The experiments fall naturally into studies of: (1) relation of the osmotic pressure of the leaves to the rate of evaporation; (2) relation of the osmotic pressure to soil temperature and soil moisture; (3) relation of the osmotic pressure to monthly precipitation and monthly mean temperature.

1. The trees tested for the relation of osmotic pressure of leaves to rate of evaporation were 4–6 feet in height and the needles were

taken at random over the tree. The results of tests made over a period of 2 months from May 22 to July 24, 1931, are recorded in table I.

Some correlation is shown between the rate of evaporation and the osmotic pressure. There is a general apparent similarity between the two curves shown in figure 1. On June 12 a drop in the rate of evaporation is followed by a drop of approximately 2.5 atmospheres in the pressure. Similarly, the marked increase in the rate of evaporation during the week of July 17 with the decrease the following week was

TABLE I

RELATION OF AVERAGE OSMOTIC PRESSURE TO RATE OF EVAPORATION

FOR WEEK ENDING	OSMOTIC PRESSURE IN ATMOSPHERES	EVAPORATION IN CC.
May 29	16.91 (4)*	474
June 5	15.76 (2)	405
June 12	14.40 (2)	No record
June 19	15.68 (4)	629
June 26	No tests	660
July 3	16.08 (2)	547
July 10	14.10 (2)	584
July 17	17.52 (2)	801
July 24	15.71 (2)	601

* The numbers in parentheses refer to the number of tests made in the week indicated.

accompanied by a marked increase and decrease in the osmotic pressure during these periods. In all probability a higher correlation would be shown if more tests were made over a longer period of time. No tests were made of evaporation for the week ending June 12, but since there was a rainfall of over 2 inches that week, it would obviously be low, and thus show a correlation with the lower pressure obtained at that time. The rainfall for the two months was 3.5 inches; thus a rainfall of 2 inches would have an effect on the evaporation for this particular week. It will be noted that the higher rate of evaporation occurred in the periods in which a higher pressure was obtained.

2. In testing the relation of osmotic pressure to soil temperature and soil moisture, leaves from trees 4–6 feet in height were used and were taken at random over the trees. The soil moisture, hygroscopic

coefficient, and pressure were determined as explained in a previous paragraph. The figures given in table II represent an average of several tests made during the weeks indicated. The data presented

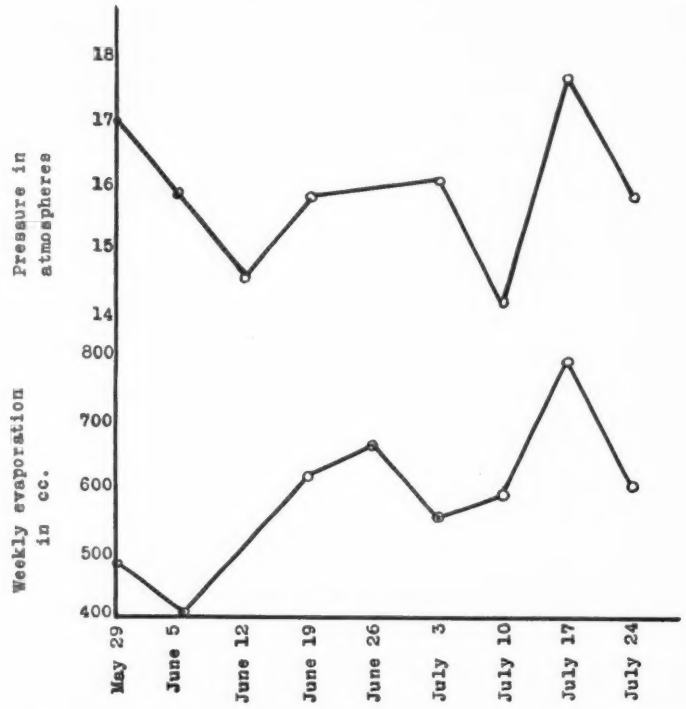

FIG. 1.—Relation of osmotic pressure of leaves of *Pinus scopulorum* to rate of evaporation. Similarity of curves is apparent, indicating a general correlation; higher pressures occur with highest rate of evaporation, and vice versa.

show no correlation between soil temperature and pressure. There is no similarity between the two curves represented in figure 2. The high pressure in May came with a low soil temperature; the maximum pressure obtained in July came with a much higher soil temperature. The pressures were approximately the same but there was a difference of 32° F. in the soil temperature. The intermedi-

ate fluctuations in pressure in no way correspond with the fluctuations in temperature during this period.

There is no definite correlation between the amount of soil moisture and the pressure. The highest pressure of approximately 17.5 atmospheres did come at a time when there was no available water

TABLE II

RELATION OF OSMOTIC PRESSURE OF LEAVES TO SOIL TEMPERATURE AND SOIL MOISTURE

Date of collection	Osmotic pressure in atmospheres		Soil temperature (°F.)		Percentage moisture	
	Individual results	Average results	Individual results	Average results	Individual results	Average results
May 22	17.37 17.48	17.42	44 44	44	32.9 24.5	28.7
May 28	16.66 16.12	16.39	56 53	54	28.3 17.8	23.1
June 6	17.14 14.39	15.82	57 58	57	33.2 29.9	31.5
June 11	14.75 14.06	14.40	63 60	62	14.5 18.0	16.2
June 15	15.14 16.26	15.70	68 66	67	26.0 21.0	23.5
June 19	15.99 15.32	15.65	68 66	67	22.2 26.1	24.2
July 3	16.71 15.45	16.08	77 73	75	21.3 11.1	16.2
July 10	14.58 13.62	14.10	78 78	78	12.1 18.0	15.1
July 18	16.68 18.36	17.52	72 76	74	9.1 6.0	7.6
July 24	15.05 16.37	15.71	78 78	78	18.6 6.4	12.5

in the soil; the moisture content was approximately 7.5 per cent. However, the high pressure of 17.4 atmospheres in the spring occurred when there was ample water available in the soil (28 per cent). A drop in soil moisture content (15 per cent) coincided with the slight drop in pressure (1.4 atmospheres) in June, but the drop in pressure of two atmospheres on July 10 came when the soil water content was 9 per cent lower than it had been during the two weeks preceding the date of collection. No definite correlation is shown in this case.

3. The determinations on the relation of osmotic pressure to monthly precipitation and monthly mean temperature were made from leaves collected from a small group of pines of approximately

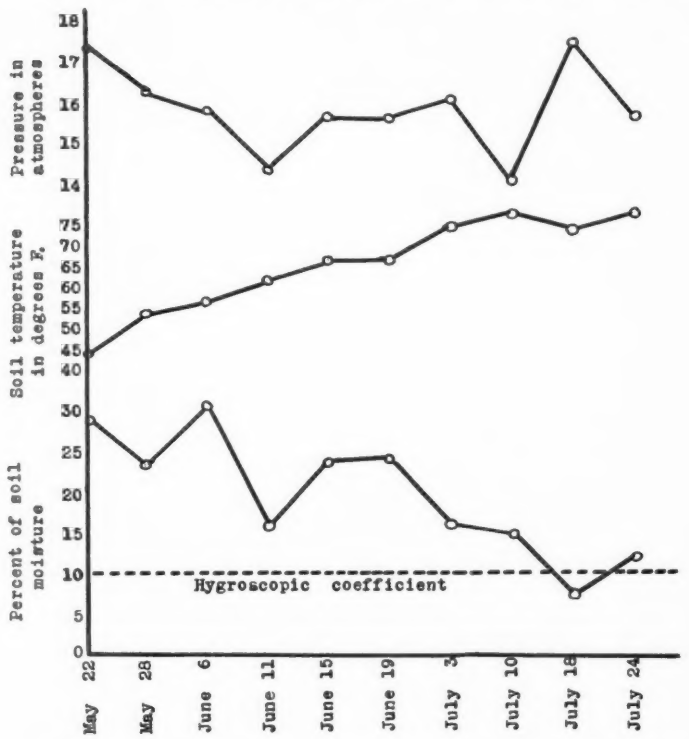

FIG. 2.—Relation of osmotic pressure to soil temperature and soil moisture, showing average of determinations of two different trees. No correlation is noted between soil temperature and osmotic pressure; no definite correlation is observed between soil moisture and pressure.

the same size, growing at the east point of Reservoir Hill. The height ranged from 10 to 15 feet. Needles were taken not more than 8 feet from the ground and at random over the tree.

No correlation is evident between the amount of precipitation and the osmotic pressure. Soil moisture tests were not made in this series.

Some correlation exists between the temperature and the pressure if we consider the monthly means for the year. There is an obvious similarity between the curves of pressure and mean temperatures in figure 3. The highest pressure of 16.7 atmospheres occurred with the

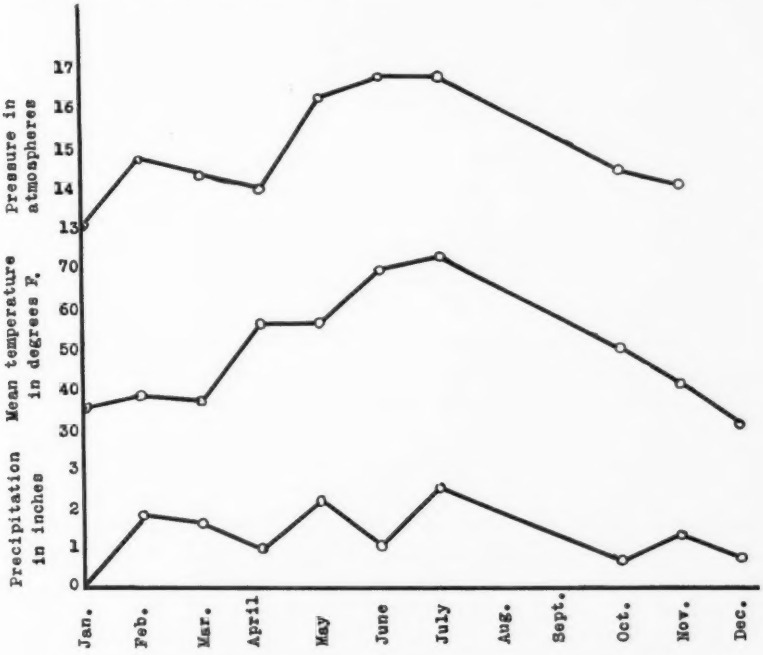

FIG. 3.—Osmotic pressure of leaves of *Pinus scopulorum*, monthly precipitation and monthly mean temperature. Little correlation is noted between amount of precipitation and pressure; direct relationship shown between monthly mean temperature and pressure.

highest mean temperature of 73°. There is also a correlation between the low mean temperature of January (35°) and the pressure at that time of 13 atmospheres.

The highest pressures in *Pinus scopulorum* for the year are not found during the dormant period in winter but are found in the summer (table III). The maximum pressure of 16.7 atmospheres was noted in July and the minimum pressure of 13 atmospheres in Jan-

uary. In each case the figures represent the average of a number of tests. In a continuation of the testing the second year the maximum pressures were found to occur again in the month of July. There was a rise in pressure of approximately 2.5 atmospheres in the month of

TABLE III

OSMOTIC PRESSURE OF LEAVES COLLECTED EAST OF CITY RESERVOIR, MEAN MONTHLY TEMPERATURE, AND MONTHLY PRECIPITATION FOR 1930

MONTH	MEAN TEMPERATURE	PRECIPITATION IN INCHES	OSMOTIC PRESSURE IN ATMOSPHERES
January	35.2	None	13.0
February	38.4	1.81 (Snow)	14.6
March	37.2	1.59 (Snow)	14.3
April	55.6	0.99	13.9
May	55.5	2.17	16.3
June	69.3	1.02	16.7
July	72.9	2.55	16.7
October	49.9	0.65	14.4
November	41.9	1.33 (Snow)	14.0
December	32.4	0.68 (Snow)	No tests*

* Leaves too dry for extraction of required amount of sap.

May, which is a period of growth. No rise in the mean temperature is evident at this time. In trees of this species occurring in this vicinity the maximum pressures are found in the summer months and the minimum in the winter months during the period of dormancy.

Discussion

This study shows a correlation between osmotic pressure and rate of evaporation, and this is in keeping with certain findings by other investigators. ENOMOTO (2) suggests that sap concentration varies directly with the temperature and inversely with the atmospheric humidity; thus the rate of evaporation would have some bearing in this case as the relative humidity of the air would affect the rate of evaporation. GILBERT and ADAMS (3) found a correlation between the total moisture in tissues and the evaporation in various crop plants. There was a depression in the total moisture of the leaves when there was a condition of high evaporation. It would appear that the rate of evaporation might be highly important in the drier habitats, owing to rapid fluctuations in relative humidity and the

difficulty with which determinations of the relative humidity can be made over a long period of time. In general, it can be stated that there is a correlation between the rate of evaporation and the osmotic pressure as shown in the data presented. When evaporation was rapid the pressure was high, and vice versa.

Correlation between the monthly mean temperatures and the monthly average of pressure was noted in one habitat over a period of one year's testing. ENOMOTO advanced the theory that sap concentration varies directly with temperature. This may be true over a long period of time and taking many determinations of sap concentration as a basis, although it was not found to be strictly true in some tests made by the writer. EATON (1) states that there is a correlation between temperature and sap concentration in leaves of some herbaceous species.

The data presented show no relationship between soil moisture and osmotic pressure of the leaves of *Pinus scopulorum*. Preliminary tests in varied habitats other than those presented here have been made in this region and no correlation has been found. Often the highest pressure is shown by trees in moist habitats. Sometimes the results are reversed in two tests, the higher pressure being found in the trees in the moist habitat one time and in the drier habitat the next time. There seems to be no reason why trees tested at the same time should show this marked variation if soil moisture, in itself, determines the pressure in leaves. In some tests an individual tree in the moist habitat showed a pressure six atmospheres above one in a dry habitat.

According to ILJIN (9), the pressure in leaves is not strictly correlated with habitat as in the case of roots; in some cases, leaves of individuals of the same species on dry habitats have a lower osmotic pressure than those in moist soil. In testing the leaves of *Pinus scopulorum* in this vicinity, it has been found that trees in a streamside habitat often have a higher pressure than those located on a rocky ridge with scant water supply. The trees in the moist habitat would also have the advantage of a lessened transpiration rate. MEYER (15) found that the osmotic value of the expressed leaf sap cannot be taken as a criterion of site over the range of habitats found in central Ohio. In experimenting with sorghum, MARTIN, HARRIS,

and JONES (13) found that the osmotic pressure of the juice was not increased by water deficiency. ILJIN, NAZAROVA, and OSTROVSKJA (10) also report that pressure in leaves gives no criterion by which to judge the pressure of roots. They found that leaves in dry places did not show a pressure higher than those in a swamp. Thus there was an inferred conclusion that there is no correlation between the pressure in leaves and the soil moisture. GILBERT and ADAMS conclude that environmental conditions have little effect upon the concentration of expressed plant solution, and that moisture in soil has no direct relationship to pressure in leaves. URSPRUNG (17) thinks that the osmotic value of the cell contents, under normal conditions, is not a measure of the power with which the cell absorbs water. He used leaves of beech trees as well as herbaceous plants for his experiments, and found no correlation between the moisture in the soil and the pressure in the leaves. WALTER and MALLERY (18) report that there is little correlation between the osmotic value of the cell sap of the leaves of *Covillea tridentata* and the moisture content of the soil. They state that the osmotic pressure of cell sap of leaves is believed to give an index of the water conditions surrounding the plant, but these conditions are so many that they must be considered as a unit rather than as separate factors.

Soil temperature has no direct effect on the osmotic pressure of the leaves of the species tested according to the determinations made in the spring and summer of 1931. Tests made over a period of two months (May 22 to July 24, 1931) showed no correlation. The maximum pressure occurred at both the maximum and minimum temperatures for that period.

In this vicinity, the highest pressures in the leaves of *Pinus scopulorum* were not found during the dormant period in winter, but in the summer. KORSTIAN (12) noted the lowest concentration in *P. ponderosa* in the winter season. He gave as an explanation the fact that fats and oils in the leaves formed emulsions with a low concentration. In the spring there is more sugar present to be used in growth, and thus there is an increase in pressure. It is obvious that there is less sap in the leaves of *P. scopulorum* in December and January since it is difficult and sometimes impossible to get enough for testing. GOLDSMITH (4) states that the pressure in young leaves

of spruce is at a maximum in early spring and then falls through the summer and winter. GAIL and CONE (5) found in the leaves of *P. ponderosa* a minimum pressure in July and a maximum in December. They think that rapid growth in April, May, and June causes lower osmotic pressure. The use of carbohydrates and other substances for growth of the new leaves and stems results in a decrease in the osmotic pressure.

In the light of the results obtained in the experiments here recorded, and of those of other workers, it seems unlikely that a definite statement can be made concerning the relation between osmotic pressure and the whole environmental complex. The great variation in pressure in the individuals of the same species, the many variable factors in the determination of pressure, and the numerous physiological factors concerned make it very difficult to state just what relationship the pressure in leaves of *Pinus scopulorum* does bear to the factors of environment.

Summary

1. A correlation was noted between the osmotic pressure and the rate of evaporation in leaves of *Pinus scopulorum*, the higher pressures generally occurring with the higher rates of evaporation, and vice versa. A difference of approximately three atmospheres was noted between the pressure occurring with the lowest and highest rates of evaporation.

2. A correlation was noted between the monthly mean temperature and the monthly averages of osmotic pressure. In general, lower pressures (13 atmospheres) occurred with the lower temperatures (35°) and higher pressures (16.7 atmospheres) with the higher temperatures (73°). The highest pressures for the year (an average of 16.7 atmospheres) were found in July, and the lowest (an average of 13 atmospheres) were found in January.

3. No definite correlation was noted between the osmotic pressure of the leaves and soil moisture.

4. No correlation was shown between the amount of precipitation and the osmotic pressure over a period of one year.

5. No correlation was shown between the soil temperature and the osmotic pressure over a period extending from May 22 to July 24, 1931.

Grateful acknowledgment is made to Dr. EDNA L. JOHNSON and Dr. FRANCIS RAMALEY, University of Colorado, for valuable suggestions and assistance during the progress of this work.

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RELATIVE TRANSPIRATION RATES IN CITRUS LEAVES[1]

A. R. C. HAAS AND F. F. HALMA

(WITH FOUR FIGURES)

In a previous paper, the writers (2) have pointed out certain physiological differences in commercial citrus species. For example, the sap of mature lemon leaves was found to be consistently more dilute and to contain less ash and calcium than the sap of orange leaves. Furthermore, on exposure to direct sunshine the sap concentration of lemon leaves was increased more rapidly than that of the orange leaves. HALMA (3) has shown that leafy cuttings of the lemon root more rapidly and produce more roots per unit leaf area than do those of either the orange or grapefruit. He has also shown that the percentage of palisade tissue in the thickness of the leaf is greater in the lemon than in the orange.

Since the photosynthetic activities are carried on almost entirely by the leaves, it seemed logical to assume that the differences mentioned are in some way related to the water requirements. BECKETT, BLANEY, and TAYLOR (1) showed that the water requirements of orange trees can be determined by means of soil moisture determinations. This method, however, would not be adequate for a study of comparative rates of water loss in different species for the reason that such a study requires leaf area measurement which in the field would be almost impracticable. Furthermore, the continuous development of new growth under orchard conditions would prevent the accurate determination of water loss per unit leaf area, because young leaves may transpire at a different rate from that of mature leaves, and one species may produce more new growth than another. Therefore, in order to determine the comparative rates of transpiration of different species, it is essential that the plants be of the same age, without new growth, and grown under identical conditions. This paper reports the results obtained for the water loss

[1] Paper no. 267, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

in three commercial citrus species, namely, Eureka lemon (*Citrus limonia* Osbeck), Marsh grapefruit (*Citrus grandis* Osbeck), and Valencia orange (*Citrus sinensis* Osbeck), together with those obtained with detached leaves of these species.

FIG. 1.—Type of citrus cutting used in solution cultures

The material consisted of cuttings which were rooted in sand by the method described by HALMA (3). Since lemon cuttings root more rapidly than those of the other two species, the former were started correspondingly later, in order to secure plants provided with comparable root systems. The orange and grapefruit cuttings were started on May 13 and the lemon cuttings on June 10. By August 7 the cuttings had well developed root systems and the leaves were in

FIG. 2.—Citrus cuttings in water cultures used for comparative transpiration studies

excellent condition (fig. 1). The roots were washed clean and the cuttings were placed in solution cultures. The culture jars were of one-quart capacity and were coated on the outside with black enamel, followed by white. Each jar contained a solution having the following composition:

PARTS PER MILLION IN CULTURE SOLUTION

Na	K	Ca	Mg	Fe	Mn	B	NO_3	Cl	SO_4	PO_4	Total
7	217	318	54	0.1	5	0.1	1211	10	216	106	2144.2

This solution had a pH value of 6.5.

Three cuttings of the same species were placed in each jar and well plugged with cotton to prevent surface evaporation. The set-up in the glasshouse as shown in figure 2 consisted of 20 jars for each species. Observations were begun on August 10 and were continued

TABLE I

AVERAGE DAILY WATER LOSS (GRAMS) PER SQUARE DECIMETER OF LEAF AREA, 1931

Species	August							August 31 to September 8	September		
	10–11	11–12	12–14	14–17	17–21	24–28	28–31		8–14	14–17	21–24
Eureka lemon.	4.64	2.78	2.43	4.66	4.91	4.50	4.79	4.07	4.70	3.44	4.65
Marsh grapefruit.....	3.98	2.43	2.09	4.18	4.64	4.41	3.72	3.10	3.26	2.85	3.25
Valencia orange.....	3.20	2.01	1.66	3.27	3.82	3.89	2.83	2.68	2.69	2.20	2.62

until September 24. Although records of temperature and humidity were kept, they are not given because all species were under the same environmental conditions. The results in table I (graphically represented in figure 3) are expressed in terms of unit leaf area.

Strictly speaking, the leaf area should be doubled, since there are two surfaces to a leaf. However, in a comparative study of this nature, where absolute values are not sought, nothing would be gained by halving the transpiration rate values. In fact, such a procedure may be misleading, since the transpiration rates of the two surfaces are unequal, as will be shown later.

It is evident that the three species show three distinct rates of daily water loss, the lemon showing the highest and the orange the lowest. The fluctuations are similar in all three curves and are related to environmental conditions.

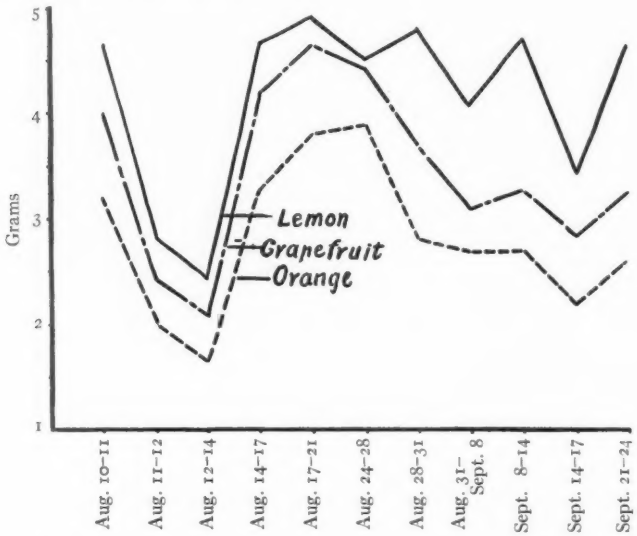

FIG. 3.—Average daily water loss (grams) per square decimeter of leaf area

An attempt was made to ascertain whether the differences in water loss in the species would be maintained under outdoor conditions. Half of the total number of jars were placed outdoors, sheltered from direct sunshine, and half were kept in the glasshouse. The average daily water loss in grams per square decimeter of leaf area for a three-day period was as follows:

	LEMON	GRAPEFRUIT	ORANGE
Outdoors	4.55	4.36	3.77
Indoors	5.53	5.18	4.89

This shows that the differences between the species were maintained regardless of environmental conditions.

These plants offered an excellent opportunity to determine the relative water losses from the upper and under sides of the leaves. The dorsal side of the leaves of six cultures of each species was coated with shellac, the ventral side of the leaves of six other cultures was likewise coated, and six other cultures served as controls. The water loss was determined twice during the period from September 28 to October 8. It will be seen from table II that there is a distinct difference between the rates of water loss of the two leaf surfaces. Notwithstanding the fact that the upper surface of citrus leaves contains no stomata, only 2 to 3.5 times as much water is transpired through the under as through the upper surface. It

TABLE II

AVERAGE DAILY WATER LOSS (GRAMS) OF UPPER AND LOWER SIDE OF CITRUS LEAVES PER SQUARE DECIMETER OF LEAF AREA

SPECIES	UPPER SIDE COATED		LOWER SIDE COATED		CONTROL		RATIO OF UPPER TO LOWER	
	SEPT. 28–OCT. 2	OCT. 2–OCT. 8	SEPT. 28–OCT. 2	OCT. 2–OCT. 8	SEPT. 28–OCT. 2	OCT. 2–OCT. 8	SEPT. 28–OCT. 2	OCT. 2–OCT. 8
Eureka lemon...	3.17	3.65	0.92	1.13	3.46	3.47	3.45	3.23
Marsh grapefruit	2.17	2.92	0.89	0.97	2.31	2.61	2.44	3.01
Valencia orange..	2.17	2.61	1.06	1.27	2.10	2.52	2.05	2.06

will also be noted that the ratio is highest for the lemon and lowest for the orange. In a similar experiment, in which detached leaves were exposed to room temperature for a period of 5 hours, the ratio was 2.5 for Eureka lemon, 2.3 for Marsh grapefruit, and 1.8 for Valencia orange.

From the discussion thus far it is clear that the three species have different transpiration rates, and also different ratios of water loss from the dorsal and ventral leaf surfaces.

Additional evidence to substantiate these differences was obtained by measuring the hourly water loss of detached leaves. Eight lots of 15 mature leaves of each species were placed in beakers containing enough water to cover the petioles and a small portion of the blade. They were then covered with bell jars lined with wet filter paper to satisfy the water deficit in the leaves. After 24 hours the leaves were wiped dry, weighed, and placed vertically on wire

screen to facilitate air circulation. The temperature in the room was 70° to 80° F. and the relative humidity was 42 to 52 per cent.

TABLE III

AVERAGE HOURLY WATER LOSS AS PERCENTAGE OF FRESH WEIGHT OF DETACHED LEAVES

HOURS	LEMON	GRAPEFRUIT	ORANGE
1	13.7	10.1	4.9
2	4.0	3.9	5.9
3	2.9	2.4	2.9
4	3.1	2.7	2.8
5	2.9	2.4	2.6
6	3.1	2.6	2.7

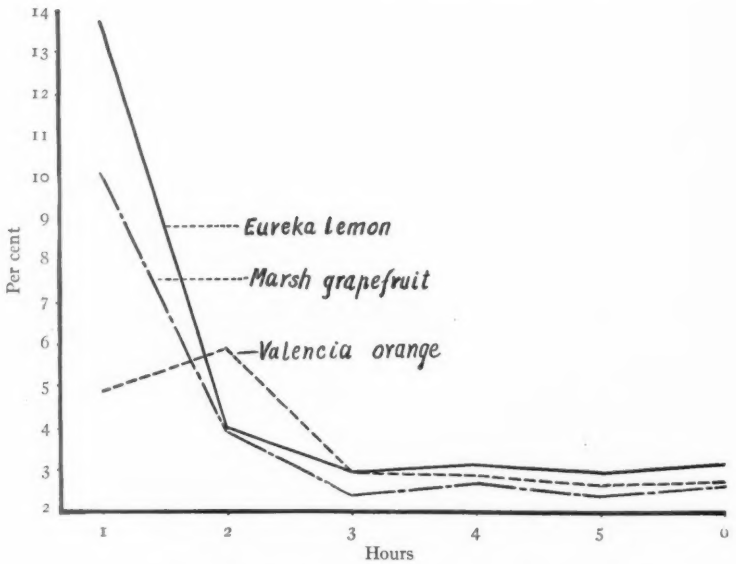

FIG. 4.—Hourly water loss as percentage of fresh weight of detached leaves

The leaves were weighed hourly for 6 hours. The results were consistent, hence only the average water loss of the eight lots for each hour is given in table III and graphically represented in figure 4.

There is a rather striking difference in the rate for the three species during the first hour between the lemon and grapefruit on the one hand and the orange on the other. Lemon leaves lost 13.7 per cent during the first hour, 4 per cent during the second, and about 3 per cent during each of the remaining hours. Grapefruit leaves lost 10.1 per cent during the first hour, 3.9 per cent during the second, and about 2.5 per cent during each of the remaining hours. Orange leaves, however, lost only 4.9 per cent during the first hour, but 5.9 per cent during the second, which distinguishes them from the other two species. The differences in the rate of water loss from these detached leaves, therefore, are in the same direction as those found for the leaves of the plants grown in solution cultures.

Summary

1. A study was made of the comparative transpiration rates of leaves of rooted Eureka lemon, Marsh grapefruit, and Valencia orange cuttings. The rate was highest for the lemon, less for the grapefruit, and least for the orange.

2. It was found that 2 to 3.5 times as much water was transpired through the under as through the upper leaf surface. There was a difference, however, in the ratio of the water loss from the dorsal and ventral sides, lemon showing the highest and orange the lowest values.

3. The water loss from detached leaves confirms the results obtained with leaves of rooted cuttings.

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LIFE HISTORY OF NYMPHOIDES PELTATUM

ERNEST L. STOVER

(WITH TWENTY-THREE FIGURES)

The present study had been completed before a reference was found to work on the gentians; later the writer was fortunate in having his attention called to the work of SCHNARF (**19**), who cites the work of STOLT (**25, 26**) on the life history of the Gentianaceae. However, it was thought worth while to publish this account, partly because the work of STOLT is little known in this country, and partly because the writer has observed some variations from STOLT's account.

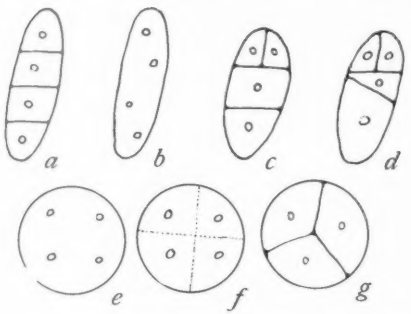


FIG. 1.—Types of megaspore formation: *a*, linear tetrad of cells; *b*, linear tetrad without cell walls; *c*, T-formed tetrad; *d*, variation of T-formed tetrad (*Diospyros*, YASUI **28**); *e*, spherical tetrad without cell walls; *f*, spherical tetrad with ephemeral cell walls; *g*, spherical tetrad with cell walls.

The species studied, *Nymphoides peltatum* (S. P. Gmel.) Britten & Rendle, has been called *Limnanthemum nymphaeoides* Hoffmansegg and Link, *Nymphoides nymphaeoides* (L.) Britton, and *Menyanthes nymphaeoides* L. In this climate it begins to bloom about June and continues blooming and producing abundant fimbriated seeds throughout the summer and until October. This species is entirely aquatic, with floating leaves and with large air chambers in both leaves and stems. The material was collected throughout two summers on the campus at Eastern Illinois State Teachers College, Charleston, Illinois. The writer feels that the plant examined belongs to the same species as that described by STOLT, judging by the descriptions and names in GRAY'S *New Manual of Botany* and BRITTON and BROWN'S *Illustrated Flora*.

The material for this study was killed in formalin-acetic acid-alcohol killing solution or in SCHAFFNER'S chrom-acetic killing fluid. Good results were obtained with both fluids.[1] After trying a number of stains, it was found that the best results were obtained by using a nigrosin-picric acid combination. This was made by mixing a 1 per cent aqueous solution of nigrosin and of picric acid half and half. This was always mixed at the time of using. When using this stain it was found best to use absolute methyl alcohol, acetone free.

STOLT summarizes his work on the gentians by pointing out that the Menyanthaceae and the Gentianaceae have cytological differences as well as differences in external appearance. His summary is as follows:

> The differences between the Gentianaceae and Menyanthaceae are not confined to such external features as position and appearance of leaves and form of vascular bundle, but show up as well in the embryo sac in the formation of the endosperm. Through these researches I have found that the difference between the families is great, as others have previously thought, and I have demonstrated further that it is authoritative to indicate the Gentianaceae and Menyanthaceae as two different families. A cellular endosperm has already been established in the order Contortae in the family Buddleiaceae (DOP 1913). Well known authors (KARSTEN 1908, ENGLER-GILLY 1912) have also included Oleaceae in this order, and I have found a cellular endosperm in *Syringa vulgaris* L.
>
> GENTIANACEAE
>
> 1. The form of the mature embryo sac is sometimes elongated (as pointed out in species of the gentians), and again oval or rounded. In the latter case it takes various forms in the course of development.
> 2. The integument tapetum is lacking.
> 3. The antipodals are often more than three in number, and in four species they are strongly developed and persistent.

[1] STOLT obtained the best result with the following killing solutions:

For young stages: chromic acid-platinum chloride-acetic acid

25 cc. of 2% CrO_3
2.5 cc. of 10% $PtCl_4$
1 cc. of conc. glacial acetic acid
75 cc. distilled water.

For older stages: zinc chloride-glacial acetic-alcohol

2 gm. $Zncl_2$
2 cc. conc. glacial acetic acid
100 cc. 50% alcohol.

He stained his material with Haidenhain's iron-alum, counterstained with light green, and Flemming's triple stain (safranin-gentian violet-orange G).

4. In the division of the primary endosperm nuclei the spindles are oriented longitudinally.

5. There is a free nuclear endosperm.

MENYANTHACEAE

1. The embryo sac is always rather narrow and elongated, often widened at the micropyle end.

2. There is an integument tapetum.

3. The antipodals are constantly three in number and degenerate quickly, often before the embryo sac is fully developed.

4. In the division of the primary endosperm nuclei the spindles are oriented longitudinally in the embryo sac.

5. There is a cellular endosperm.

As to the development of antipodals, STOLT reports that in the Menyanthaceae they are apparently three and ephemeral. His summary of the antipodals in gentians follows:

A. The number of antipodals constantly three:
   1. Uninucleate normally developed antipodals: *Exacum affine*, *Chlora perfoliata*, *Erythraea centaurium*, *Gentiana pneumonanthe*, *G. ciliata*, *G. crinita* Frön., *Voyria coerulea*.
   2. Multinucleate, very large antipodals: *Halenia elliptica*.

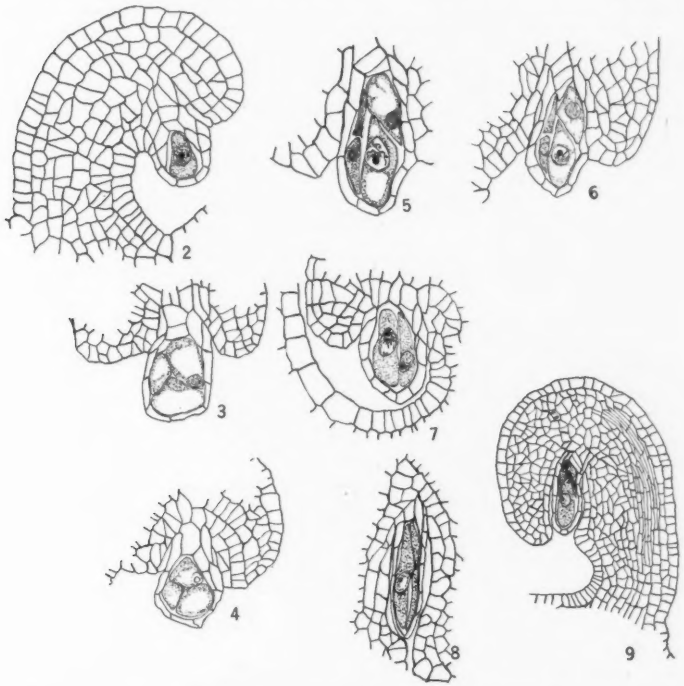
B. The number of antipodals varying three or more, weakly developed:
   Gentiana lutea, *G. asclepiadea* L., *G. nivalis*, *G. tibetica*, *G. walujewi* Rgl., *G. straminea* Max.

C. The number of antipodals always more than three and well developed:
   1. Th number always six:
      Gentiana tenella.
   2. The number always greater than six:
      Gentiana amarella, *G. uliginosa*, *G. germanica*, *G. campestris*.

In the life history of *Nymphoides peltatum*, as recorded by STOLT, the megaspore mother cell protrudes from the ovule and there is no tapetal cell. The megaspores are arranged in a linear tetrad. The integument grows around the persisting megaspore and the cells of the nucellus that inclose the megaspore are absorbed by the time the four nucleate embryo sac is formed. The normal eight nucleate embryo sac is developed and both fertilization and triple fusion occur. The fertilized egg elongates and before it divides the embryo sac is completely filled with a cellular endosperm. The embryo is dicotyledonous and has a weakly developed suspensor.

Megaspore formation

This account varies from that by Stolt in that the megaspores are in a well demonstrated spherical tetrad (figs. 3–8); and a suspensor of only one cell was found to be developed in the embryo, the

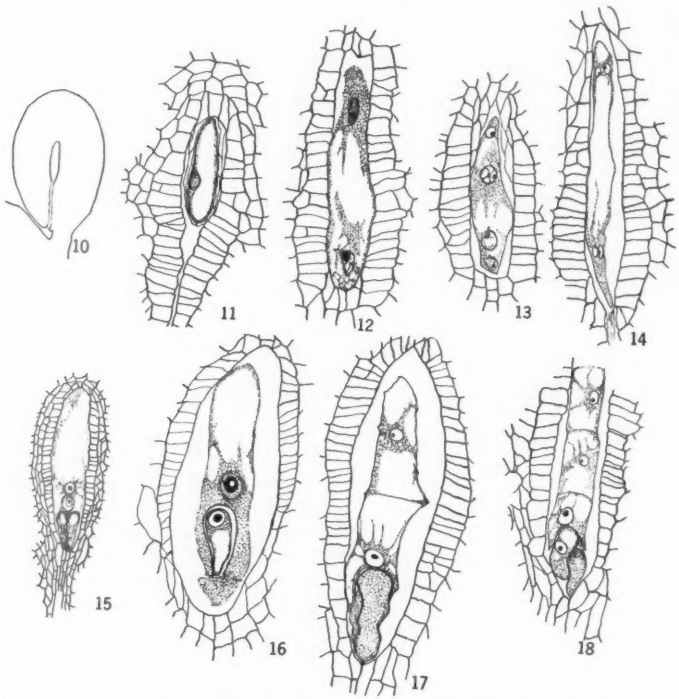


Figs. 2–9.—Fig. 2, protruding megaspore mother cell; ×215. Figs. 3–8, various views of spherical tetrad of megaspores; ×500. Fig. 9, ovule showing growth of integument about persisting megaspore and remains of two megaspores; ×100.

largest suspensor seen being illustrated in figure 23. The ovules during their development change their positions in the ovary, so that perfect sections of all stages were very difficult to obtain.

The megaspore mother cell protrudes from the ovule (fig. 2) and there is no tapetal cell. Reduction division results in a spherical tetrad of megaspores as shown in figures 3–9. As three of these mega-

spores are being absorbed, the fourth enlarges and the integument grows down around the persisting megaspore until it occupies approximately the center of the ovule (fig. 10). The epidermal cells



FIGS. 10–18.—Fig. 10, diagram of ovule showing position of embryo sac. Fig. 11, megaspore with disintegrating jacket cells and surrounding integumentary tapetum; ×500. Figs. 12–14, two and four nucleate embryo sacs; ×500. Fig. 15, embryo sac ready for fertilization; ×215. Figs. 16–18, 1-, 2-, and 4-celled endosperms with fertilized egg (fig. 18 also shows remains of pollen tube); ×500.

that inclose the megaspore are next gradually absorbed (fig. 11) and have usually disappeared by the time the two nucleate embryo sac is formed (fig. 12).

The first summary of the variations in megaspore formation was made by COULTER (**3**) in 1908, in which he pointed out that the megaspores do not always form a linear tetrad of cells. In the case of *Lilium* and its allies no cell walls are formed, and in the Orchida-

ceae and Piperaceae other variations have been found. The next summary of the subject was made by PALM (**16**), who pointed out three types: the linear tetrad, the T-formed tetrad, and lastly the quadrant-formed tetrad.

In view of this study a complete classification of types of megaspore formation is listed as follows:

A. Linear tetrad
   1. with cell walls: *Capsella* et al.
   2. without cell walls: *Lilium*.

B. T-formed tetrad:
   Diospyros (YASUI); *Trillium* (HEATLEY); *Trillium* (SPANGLER); *Thismia* (PFEIFFER); *Gasteria* (STIFFLER).

C. Quadrant or spherical tetrad:
   1. without cell walls: Orchidaceae (SHARP); *Peperomia* (JOHNSON, BROWN).
   2. with ephemeral cell walls: *Smilacina* (MCALLISTER).
   3. with cell walls: *Nymphoides*.

This apparently exhausts all the possibilities as to kinds of megaspore arrangement.

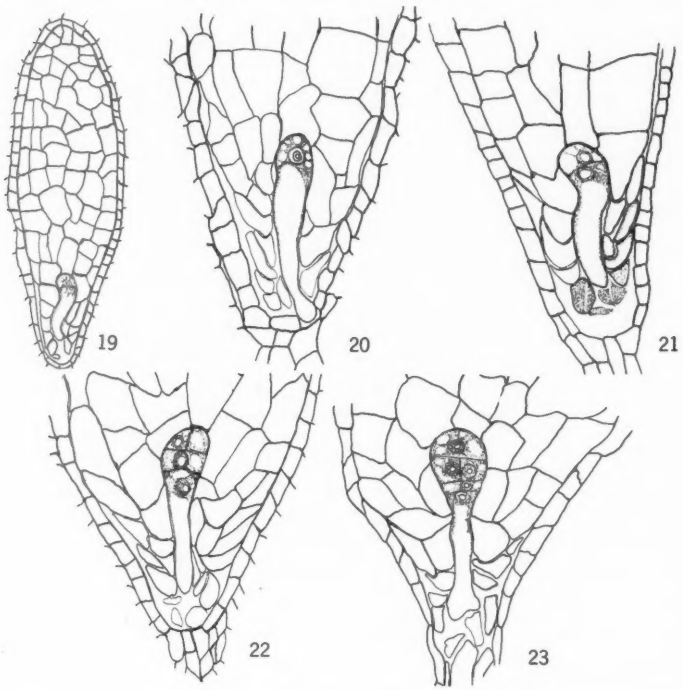
EMBRYO SAC

The inner cells of the integument form an integument tapetum (described by PALM) (figs. 11–18), and the embryo sac is held in the ovule without any surrounding nucellar cells, directly exposed to the micropyle (figs. 12–18). The "normal type eight nucleate embryo sac" (SCHNARF) is formed, although it was not found in one perfect section. The mature embryo sac consists of four cells: the egg, the endosperm (fusion) nucleus, and the two elongated synergids. There are no antipodals at that stage, as they are represented only by nuclei which have disintegrated (fig. 15). The pollen tube enters the ovule through the micropyle in all cases observed, and the end of the pollen tube often persists for some time (fig. 18). After fertilization the egg elongates with its haustorial end toward the micropyle (fig. 16).

ENDOSPERM AND EMBRYO

The endosperm cell becomes very large and surrounds the fertilized egg before it divides (fig. 16). Figures 17 and 18 show two-

celled and four-celled endosperms. The finding here agrees with that of STOLT, who states that he is sure that there is no free nuclear division of endosperm, although he did not see these early stages. Division continues until the endosperm completely fills the embryo



FIGS. 19–23.—Fig. 20, elongated fertilized egg with endosperm completely filling embryo sac. Figs. 19, 21–23, stages in development of young embryo; ×215.

sac around the undivided fertilized egg (fig. 20). This development of the endosperm before mitosis in the egg has been observed by SHOEMAKER **21**, SHREVE **22**, GOW **6**, and EDWARDS **4**, in *Hamamelis*, *Sarracenia*, *Arisaema*, and *Hedyosmum*.

After the growth of the endosperm the fertilized egg divides and one cell becomes the elongated haustorium-like cell toward the micropyle, and the other by subsequent divisions becomes the em-

bryo proper (figs. 19, 23). The embryo absorbs some of the endosperm cells and continues in its growth until the ripe seed consisting of embryo, endosperm, and testa is fully developed.

Inasmuch as this plant is not a native of this country, the variations here described for the behavior of the megaspore and the absence of the suspensor may be caused by the environment. Although there are no records citing such variations in these structures, it seems reasonable to suppose that they may be the effects of external conditions; or it may be a slight mutation.

Summary

1. The megaspore mother cell of *Nymphoides peltatum* protrudes from the ovule and there is no tapetal cell.

2. The result of reduction division is a spherical tetrad in the ovule.

3. The embryo sac is not surrounded by a nucellus but lies next to the integument.

4. There is an integumentary tapetum.

5. The embryo sac is the normal type of eight nuclei.

6. The antipodals are ephemeral.

7. The pollen tube enters the ovule through the micropyle.

8. The endosperm is cellular and there is no free nuclear division.

9. The fertilized egg does not divide until the endosperm has filled the embryo sac.

10. The embryo has an ephemeral one-celled suspensor.

11. The mature seed contains a dicotyledonous embryo imbedded in an endosperm.

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COMPOSITION OF THE FIBER AND WASTE OF AGAVE LECHUGUILLA

ROBERT A. GREENE

Introduction

The agave family, consisting of some 300 species, is found chiefly in the desert regions of Mexico and the southwestern part of the United States. In this country the agaves are of interest principally to botanists and plant collectors. In Mexico, their native home, they furnish fiber, soap, and are the source of three Mexican drinks, pulque, mescal, and tequila. BAILEY (**2**) has given a brief but concise description of the principal species and their importance, and TRELEASE (**14**) has contributed an article on the same subject.

Agave lechuguilla Torrey is found in western Texas and along the Rio Grande as far east as Presidio; it extends into New Mexico and Mexico, where it grows abundantly, particularly in the states of Tamaulipas, Chihuahua, Coahuila, Hidalgo, and Nuevo Leon (**13**). It is a very hardy plant with a low water requirement.

This species furnishes a fiber which is called ixtle or tula. The fiber is secured from the leaves of the plant, and in the preparation of ixtle approximately 60 per cent of the plant is left as waste. A small amount of this is used for cleansing purposes, and still more is used for fuel, but at present it has no commercial value. This investigation was undertaken in the endeavor to find some commercial use for this waste. The problem was suggested by Mr. WILLIAM RICHARDSON of Hacienda del Rosario, Parras, Coahuila, Mexico, and this paper is only a preliminary report.

Experimentation

Approximately 10 kg. each of ixtle and of the waste were collected by Mr. RICHARDSON, from the vicinity of Parras, Coahuila. These samples were composites and were taken from several sources, so that they may be considered as representative. At the time the samples were collected they were fairly dry. Upon arrival at the laboratory, they were air-dried, divided into quarters and about 1 kg. of each finely ground. All analyses were made upon these two samples.

In order to secure some idea of the general nature of these materials, they were subjected to the system of analysis commonly used

TABLE I

Material	Percentage	
	Waste	Fiber
A. Feed analysis (air-dry basis)		
Moisture	7.87	5.79
Ash	8.97	1.55
Protein	4.31	0.94
Crude fat	2.66	0.84
Crude fiber	16.80	63.30
Nitrogen-free extract	59.39	27.58
B. Carbohydrates (air-dry basis)		
Reducing sugars (as dextrose)	7.68	0.91
Invert sugars	6.52	1.31
Starch	3.36	1.16
Galactans	2.21	0.77
Pentosans	7.63	19.05
C. Proximate analysis (moisture-free basis)		
Ether-soluble fraction	2.97	0.89
Alcohol-soluble fraction	1.88	1.01
Cold water-soluble organic matter	43.49	4.01
Hot water-soluble organic matter	3.51	0.80
Hemicelluloses	6.32	18.84
Celluloses	6.82	41.64
Lignins	10.05	15.44
Crude protein	3.03	0.45
Ash	9.68	1.65
Total	87.75	84.73
D. (air-dry basis)		
Nitrogen	0.69	0.15
Phosphorus (as P_2O_5)	0.15	0.06
Potassium (as K_2O)	1.27	0.28

for feeds. The methods used were those of the Association of Official Agricultural Chemists (1) and the results of the analyses are given in table I, A.

These analyses showed that the waste contained a rather large amount of carbohydrates, so a more complete analysis was made.

The Official Methods (1) were used in all determinations, the only deviation being in the determination of starch. Taka-diastase was used for hydrolysis rather than freshly prepared diastase. This method, suggested by HORTON (9), gives satisfactory results and saves considerable time by eliminating the preparation of diastase. The results of these analyses appear in table I, B.

The so-called crude fiber of the feed analysis is a mixture of cellulose, a part of the hemicelluloses and lignin, as well as other substances which make up the framework of the plant. In order to secure some information concerning these constituents, a proximate analysis was made, using the method of WAKSMAN and STEVENS (16). By the use of this method it is usually possible to account for 88 to 95 per cent of the material. The results of these determinations are given in table I, C.

Table I, A, shows that the nitrogen content of the waste is approximately the same as that of wheat straw, hay, or grama grass, but that it has a rather high ash content. Consequently phosphorus and potassium were determined, and the percentages of these elements are given in table I, D.

Discussion

Table I, A, does not reveal anything especially significant. The general composition of the waste is similar to that of Hegari fodder, brome grass, prickly pear stems, dry cholla fruit, grama grass, and several other forages analyzed by CATLIN (7). The sample of fiber is characterized by a high crude fiber content, as might be expected.

No attempts were made to identify the individual sugars which appear in table I, B, although glucose is probably the most abundant constituent. This sugar was tentatively identified by the saccharic acid test in the cold water and hot extracts, and by the hydrolysate of the hemicelluloses and celluloses (table I, C). Galactans were determined by oxidation to mucic acid. Although qualitative mucic acid tests indicated that there was some galactose in the water-soluble extract, yet probably the larger part occurs as a galactan. The water-soluble galactose possibly results from a partial hydrolysis of the saponin which JOHNS, CHERNOFF, and VIEHOEVER (10) have

found in *Agave lechuguilla*. This saponin, which is found in all parts of the plant, in the rootstocks as well as the leaves, is soluble in water and alcohol, and is not precipitated by lead acetate or lead subacetate. Upon hydrolysis, glucose and pro-sapogenin are formed; further hydrolysis gives galactose and an end-sapogenin, whose formula is $C_{15}H_{24}O_2$. It is apparent that this saponin would appear in the sugar analysis, since it would not be removed in the process of clarification. It is probable that the reducing sugars found in the fiber are due to impregnation with this saponin, which either occurs in the fiber or was absorbed from other parts of the plant during the separation of the fiber from the remainder of the leaf. As considerable time elapsed after the time of this separation, it is probable that the saponin was hydrolyzed to a considerable degree, either by enzymes, bacterial action, or the natural acidity of both the fiber and waste.

Qualitative tests for starch were negative, indicating that the sugars resulting from the action of diastase were formed by the hydrolysis of dextrin or some carbohydrate material other than starch. As a general rule, however, monocotyledons do not store starch.

Unfortunately, analyses of this type are not so clear cut as inorganic determinations. Different methods give entirely different results, and at best there is always some overlapping of groups of constituents. This is reflected in the results for pentosans (table I, B) and hemicelluloses (table I, C). When the limitations of the analytical methods are considered, however, the results agree very well. The former are determined as phloroglucides and the latter by acid hydrolysis and subsequent determinations of reducing sugars. The composition of hemicelluloses varies with different plants. They are condensation products of hexoses, pentoses, or both with uronic acids. Upon hydrolysis they give simple sugars, hexoses, or pentoses:

$$(C_5H_8O_4)n + nH_2O = n(C_5H_{10}O_5)$$

$$(C_6H_{10}O_5)n + nH_2O = n(C_6H_{12}O_6)\ .$$

The pentosans are the most abundant group of hemicelluloses, and according to WAKSMAN (**15**) vary from 7 per cent of the total constituents of pine needles to 32 per cent in corn cobs. The formation of

phloroglucides, however, is recognized as not being entirely specific for pentoses. The treatment with 12 per cent hydrochloric acid will convert galacturonic acid to arabinose, and then to furfural. Consequently a hemicellulose containing galacturonic acid would give the same results as though xylose or arabinose were present. Hemicelluloses usually consist of mannose, galactose, arabinose, xylose, and occasionally fructose and glucose. It appears, however, that so far as the methods are applicable, the hemicelluloses of both the fiber and waste consist principally of pentosans.

The nitrogen-free extracts of the waste and fiber are 59.39 and 27.58 per cent respectively. Only about 47 per cent of this amount in the waste and 84 per cent of the amount in the fiber are accounted for by the sugar analysis. The remainder probably occurs as acids, esters, and other compounds.

In the proximate analysis, approximately 85 per cent of the plant constituents are accounted for. These low results are probably due to the fact that the more complex carbohydrates (especially the compound celluloses) are not determined by this method. In nature, cellulose appears in many compound celluloses. BERNTHSEN (**4**) gives the following examples: ligno-cellulose, a combination of lignin and cellulose, which is found in jute and the lignified tissues of perennial stems; pecto- and muco-celluloses; adipo- and cuto-celluloses. Pectocelluloses, which yield pectin or pectic acids and cellulose upon mild hydrolysis, are found in the flax fiber and in China grass. In cork the cellulose is "combined or mixed with cutin and suberin, which appear to be glycerides of complex acids, such as stearo-cutic."

In view of the complex structure of plants, it is remarkable that by such methods, usually empirical, it is possible to account for such a large percentage of the constituents.

The waste and the fiber, when dissolved in water, both gave solutions which were acid to litmus. The acid occurring in *Agave americana* has been reported as oxalic by D'HERELLE (**8**), as lactic by MCGEORGE (**17**), and as malic by ZELLNER (**18**).

Since considerable time had elapsed since the samples were collected, and since some acids may have been formed by bacterial action, or since the acid originally present in the plant might have been decomposed, the acid was not definitely identified.

Total acidity of these materials was determined by shaking 10 gm. of material with 250 cc. of water, filtering, and then titrating with NaOH, using phenolphthalein as an indicator: 2.57 cc. of tenth normal HaOH were required to neutralize the acids present in 1 gm. of waste and 0.36 cc. for 1 gm. of fiber.

Another aliquot was evaporated on a steam bath to a thick syrup but no crystals were obtained. Crystals could not be obtained from the evaporation of alcohol or ether extracts. When $CaCl_2$ solution was added to a small amount of the water extract, a white precipitate formed which dissolved upon dilution with water. These results, as well as solubility tests (using the scheme commonly employed in organic qualitative analysis), indicated that the principal acid present might be lactic rather than oxalic or malic, although lactic acid was not the only acid present in the sample analyzed. The total acidity of the waste calculated as lactic acid was 2.25 per cent.

Commercial possibilities

The results suggest several possible industrial uses for both the fiber and waste, but it is extremely doubtful whether any of these would be profitable under present economic conditions. Among these might be the use for making paper pulp (as suggested for waste of *Agave americana* by LEIGHTON **12**), the preparation of furfural from the pentoses of the fiber, the isolation of the lactic acid (providing it is present in fresh material), or local use of the waste as stock food, providing the toxic saponin were removed.

The fiber and waste were found to be only slightly soluble in some of the usual cellulose solvents (cuprammonium solution, or zinc chloride), so they are not suitable for use as raw materials for the production of artificial silk or other cellulose products.

The production of alcohol or other products by fermentation of the carbohydrates seems to be the best method of utilizing the waste. A small quantity of ethyl alcohol was obtained in this laboratory by neutralizing an aqueous extract of the waste with $CaCO_3$, and fermenting with ordinary baker's yeast. Although the yield was small the results were encouraging. Owing to the technical difficulties involved (bulk of the waste, removal of the saponin, as well as the relative abundance of readily fermentable materials such as molasses,

which are a drug on the market), however, it is doubtful whether alcohol production would be profitable. Its production from sisal waste has been discussed by D'HERELLE (8), LAVEDAN (11), BRUNO and SORGES (6), BOULARD (5), BAUD (3), and others, but at present there is no commercial production of alcohol from this source.

Both the waste and fiber contain small quantities of nitrogen, potassium, and phosphorus, so that these materials might have some local use as fertilizers. The writer agrees with MCGEORGE (17), that under present conditions the best utilization of the waste is to return it to the soil as fertilizer. In this way only the mineral elements in the fiber are lost to the soil.

The analytical data are in agreement with those of sisal waste (MCGEORGE 17) and of the whole leaf of *Agave americana* by ZELLNER (18), which would indicate a similarity in composition.

Summary

1. The fiber and waste of *Agave lechuguilla* have been analyzed and found to be similar in composition to those of *A. americana*.

2. Under present conditions there appear to be no profitable industrial uses for either the fiber or the waste.

3. If methods were developed to manufacture alcohol profitably from sisal waste, that of *A. lechuguilla* could be used for the same purpose.

4. Locally the waste might be used as a cattle food by removing saponin. Probably its best use is in its return to the soil as a fertilizer.

The writer wishes to express his indebtedness to Dr. P. S. BURGESS, Dean of the College of Agriculture, whose kindness has made this study possible; to Mr. W. Y. GARY, who made some of the determinations; and especially to Mr. WILLIAM RICHARDSON, Hacienda del Rosario, Parras, Coahuila, Mexico, who suggested the problem and supplied the materials.

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CURRENT LITERATURE

BOOK REVIEWS

The cacti

The almost simultaneous appearance of two books concerned with the same group of plants and written in the same city might lead the reader to expect much duplication. Fortunately the books prove to be complementary and abound in diversities rather than in repetitions. The first, by SHREVE,[1] is an ecological description, as might be surmised from the title. Its chapter headings: "What the cactus is; How the cactus is built; What the cactus is named; The cactus in its home; and How to cultivate the cactus," give some idea of the volume's scope. Written in non-technical language, but scientifically correct, the various chapters give an excellent picture of the fundamental problems of plant life in arid regions, and show how some of these problems have been solved by this family of plants. The practical application of the ecology of the cactus is seen in directions for the cultivation of these interesting plants.

Problems of distribution are presented in an interesting manner, and the reader may learn from the appendix in what lands the 1235 species may be found. Surprisingly enough, only one-sixth of the species belong to the United States, while Mexico and South America each have about twice as many.

The second cactus book, by THORNBER and BONKER,[2] surpasses the first in its wealth of illustrations and in its emphasis on the strange and the fantastic features of the family. While it is also non-technical, the plan of the book is different too, for its attention is focused upon individual species, and the reader is told how to identify the plants, what fantastic qualities they possess, and how to cultivate them. The illustrations include photographs, clever drawings, and excellent reproductions of beautiful paintings.

It is hard to imagine a better pair of books fitted both to interest the layman and to instruct the botanist in a group of plants that is increasing in popularity. Both books are well printed and attractively bound.—G. D. FULLER.

Trees of Indiana

In few of our states are the details of the distribution of plant species more thoroughly known than in Indiana. This is due largely to the persistent efforts of DEAM, who has devoted more than a quarter of a century to this study. The

[1] SHREVE, FORREST, The cactus and its home. pp. vii+195. *Figs. 43.* Williams and Wilkins Co. Baltimore. 1931. $3.00.

[2] THORNBER, J. J., and BONKER, FRANCIS, The fantastic clan. The cactus family. pp. xiv+194. *Figs. 53, map.* The Macmillan Co. New York. 1932. $3.50.

continuation of his investigations is indicated by the appearance of a second revised edition of his *Trees of Indiana*,[3] which is much more than a reprint of former editions, the first of which appeared in 1911. The numerous illustrations made from specimens in DEAM's herbarium and the distributional maps greatly enhance the value of the book. The care exercised in the preparation of the text, the great number of illustrations, the excellence of the typographical work, and the amount of original material, all go to make it one of the best tree books yet published by a state.—G. D. FULLER.

Microchemistry

Since its publication in 1913, TUNMANN'S volume on microchemistry has been one of the leading text and reference books in this field. ROSENTHALER is the author of the second edition[4] of this well known book. The second edition follows the first in the arrangement of parts, but is very much larger, containing 1047 pages as compared with 631 pages in the first edition. The increase is rather uniformly distributed throughout the book, and consists of additions to the methods, references to recent literature, and new sections. Among the new subjects treated are micromanipulation, acetaldehyde, urea, vital staining, membrane pigments of the mosses, pigments of the molds and bacteria, and microchemistry of yeast and bacteria. The first edition contains 137 illustrations; the second, 190.

The volume is too large and expensive for a textbook, but is invaluable as a reference book for the botanist, pharmacologist, and anyone interested in microchemical methods.—S. V. EATON.

The beech forests of Europe

As early as August, 1923, the ecologists of Europe, assembled on their International Phytogeographic Excursion, decided to collect data on the ecology and phytogeography of the beech (*Fagus silvatica*) forests of Europe. Two years afterward their plans appeared in print,[5] in order that the resulting contributions should achieve some degree of uniformity in presentation and that they might result in a composite picture of European beech woods.

The project received still further impetus from an invitation excursion initiated and conducted by TANSLEY in connection with the International Botanical Congress at Cambridge in 1930. Before the ecological section of this Congress a symposium on beech forests was presented and papers were read by twelve

[3] DEAM, C. C., Trees of Indiana. pp. 326. *Pls. 140; 118 maps.* Department of Conservation, State of Indiana. Published in Fort Wayne. 1931.

[4] TUNMANN, O., Pflanzenmikrochemie. Ein Hilfsbuch beim mikrochemischen Studium pflanzlicher Objecte. 2d ed. by L. ROSENTHALER. *Figs. 190.* Gebrüder Borntraeger. Berlin. 1931.

[5] RÜBEL, EDWARD, Vorschläge zur Untersuchung von Buchenwäldern. Beiblatt Veröffen. Geobot. Inst. Rübel Zürich 3:1–35. 1925.

European ecologists. These papers, published in abbreviated form in the Proceedings of the Congress,[6] have been expanded and now appear with four other contributions in an exhaustive monograph.[7]

An introduction and summary are supplied by RÜBEL, who has edited the collection. The following authorities have discussed the characteristics of the forest within their respective countries: MARKGRAF of Germany, DOMIN of Czechoslovakia, SZAFER of Poland, STOYANOFF of the Balkan Peninsula, BORZA of Rumania, WULFF of the Crimea, UELINGER of Switzerland, OSTENFELD of Denmark, LINDQUIST of Sweden, WATT and TANSLEY of England, VIERHAPPER of Austria, and CUATRECASAS of the Iberian Peninsula. In addition ISSLER has described the occurrence of the beech in subalpine regions and CZECZOTT has given in detail the distribution of *Fagus orientatis* Lipsky.

In all cases the authors give the range of climatic factors within which beech-wood occurs in their particular countries, also the altitudinal limits of the tree and the soils upon which it develops. Attention is also given to the varieties of forest management to which the beech has been subjected by man and to the past history of forests. It is recognized that the beech tolerates a wide range of conditions, and that it is unaffected by minor changes of climate, soil, and biotic influence, although such changes effect differences in the floristic composition of the undergrowth which is associated with it.

It is upon the basis of the species constantly appearing in the various beech forests that RÜBEL divides the aggregate (Fagion silvaticae) into ten associations: Fagetum asperulosum, Fagetum alliosum ursini, Fagetum melicosum, Fagetum mercurialosum, Fagetum caricosum, Fagetum altherbosum, Fagetum filicosum, Fagetum ericaceosum, Fagetum südalpines, and Fagetum orientalis. Each of these association types is briefly characterized in the summary of the symposium. Bibliographies, association tables, illustrations, and diagrams add to the value of the monograph.—G. D. FULLER.

Contribution to Tertiary floras

Our knowledge of the Tertiary flora of Poland receives a valuable augmentation through several publications by a Polish paleobotanist.[8] These papers describe new fossils which have been found in the Tertiary of Poland and include a general discussion of the development of ecological conditions of the Tertiary floras.—A. C. NOÉ.

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[7] RÜBEL, E., Die Buchenwälder Europas. Veröffen. Geobot. Inst. Rübel Zürich **8**:502. Hans Huber. Bern and Berlin. 1932. Fr. 25.

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